ACTION SPECTRUM FOR THE PHOTOPERIODIC CONTROL OF FLORAL INITIATION OF SHORT-DAY PLANTS

M. W. PARKER,[1] S. B. HENDRICKS,[2] H. A. BORTHWICK,[3] AND N. J. SCULLY[4]

Introduction

It has been shown that floral initiation in short-day plants can be prevented by brief periods of illumination applied near the middle of the dark period (**15**). The interruption, depending upon the light intensity, need not be more than a few seconds. A study of the effectiveness of different wave lengths of light on this reaction has been made, and curves relating wave-length to effectiveness, which may be referred to as the action spectrum, have been constructed. Only by means of quantitative data which can be expressed in the form of an action spectrum curve can information be obtained as to the fundamental nature of the photoreactions that are responsible for the photoperiodic responses of plants. Many investigators, all using filtered radiation, have studied the influence of various regions of the spectrum on this reaction, but none has made quantitative determinations of the relative effectiveness of the different wave-length bands.

Four conditions were established in experiments for determining the action spectrum. First, use was made of the inhibiting action on floral induction of brief periods of irradiation applied at or near the middle of the dark period. Second, the radiation applied at these times consisted of relatively narrow wave-length bands of high spectral purity. Third, the source of radiant energy for these dark-period interruptions was of high intensity in all regions of the visible spectrum. Fourth, the biological response investigated was restricted to floral initiation.

[1] Physiologist; [2] Senior Chemist; [3] Senior Botanist; [4] Associate Physiologist; Bureau of Plant Industry, Soils and Agricultural Engineering, Agricultural Research Administration, U.S. Department of Agriculture, Plant Industry Station, Beltsville, Md.

Review of literature

RAZUMOV (**16**), working with both long-day and short-day plants, concluded that radiation from the red and yellow parts of the spectrum, when used to extend short natural photoperiods, was equivalent to daylight in its effect on the photoperiodic reaction, whereas green, blue, or violet radiation was equivalent to darkness. Both KATUNSKIJ (**10**) and KLESHNIN (**11**), on the contrary, found that the photoperiodic reaction could be influenced by radiation from any part of the visible spectrum but that the intensities required differed from one region to another. KATUNSKIJ reported that red was most effective, with yellow-orange a close second and blue third in effectiveness. He found green to be the least effective region and noted that there was a close similarity between photoperiodism and photosynthesis in their dependency on the spectral composition of light. KLESHNIN called special attention to the fact that it was necessary to use low intensities of radiation if differences in effectiveness of different spectral regions were to be distinguished, and he suggested that failure of some workers to consider this fact probably accounted in part for contradictory conclusions.

WITHROW and BENEDICT (18) investigated the effects of several different wave-length bands and found that orange and red resulted in earliest blooming and greatest number of flowers with pansy, stock, and aster. Blue, green, and yellow radiation had little effect on pansy and stock, but with aster all wave-length bands were effective in hastening flowering. WITHROW and BIEBEL (19) extended these investigations to include short-day plants and found that the red wave-length band, which was most effective in promoting flowering of long-day plants, was likewise most effective in preventing flowering of short-day plants. WITHROW and WITHROW (20) carried out further work with red radiation and studied the effects of blue light in greater detail. They were aware that small energies of red radiation were sufficient to produce the effects observed and were not certain that in previous experiements these levels had not been exceeded by extraneous unfiltered light. Their results showed almost no effect of blue radiation and confirmed previous observations as to the effectiveness of the red region. They concluded that the leaf pigment controlling the photoperiodic response of all plants with which they worked absorbs strongly in the red, less so in the yellow-green, and least in the blue regions of the spectrum.

Several experimental conditions could have contributed to the diverse results obtained with blue radiation. First, these studies were made with filtered radiation, and the filters used by the different workers were not identical. In most cases they transmitted very wide wave bands, and some authors appear to have attached insufficient importance to the weak transmission that may have occurred in wave bands outside the principal region of transmission. Second, most workers failed to employ energy levels near the threshold required in the different wave bands to produce the photoperiodic effect. The energy in the red was frequently higher than necessary and in the blue was too low to produce an effect. Third, the biological response measured could have been more sharply defined in many cases. Basically, all workers were concerned primarily with the spectral conditions that permitted transition of the plants from a purely vegetative development to a reproductive one. The effectiveness of a given treatment was in nearly all cases judged by the ultimate formation or lack of formation of macroscopic flower buds or open flowers. This procedure required that experimental treatments be of several weeks' duration. It assumed that the plants had not initiated flower primordia before the treatments were applied and further assumed that the effectiveness of a given treatment in causing floral initiation was similar to its effectiveness in promoting the subsequent growth and development of these flower primordia into macroscopic buds and flowers. This assumption is not valid in the case of soybean, and probably many other plants would have similar behavior. Soybean plants with established flower primordia can be given photoperiods that will prevent their development into flowers or fruit.

Biological material and experimental procedures

The short-day plants used in this investigation were soybean, *Soja max* (L.) Piper var. Biloxi, and cocklebur, *Xanthium saccharatum* Wallr. The plants were grown in soil in $3\frac{1}{2}$- or 4-inch pots in the greenhouse and were produced in sufficient number so that very uniform populations could be selected for each experiment.

All experiments with Biloxi soybean by this project for the past several years have been conducted with seed derived from the same original source, new crops of which have been produced from year to year. This strain produces very uniform plants and contains no types that vary significantly from the rest in their photoperiodic behavior. Most of the cocklebur seed was obtained along a road near the grounds of the Bureau of Plant Industry Station. For preliminary experiments, however, seed was obtained through the courtesy of Dr. E. J. KRAUS from the source used by HAMNER and BONNER (7) and referred to by them as *X. pennsylvanicum*. The photoperiodic responses of the plants from the two sources were identical, and both were determined by Dr. S. F. BLAKE as *X. saccharatum* Wallr.

Because of the limited amount of leaf area that could be irradiated with the spectrograph, it was necessary to reduce the leaf surface by defoliation as much as was possible without interference with the capability of the plant to respond to photoperiodic treatment. With soybean all expanded foliar organs except the terminal leaflets of the third compound leaf were detached. It had been found in earlier studies (3) that initiation of flower primordia by plants defoliated in this way was not impaired. With cocklebur all leaves but the most recently fully expanded one on each plant were removed. The leaf irradiated was usually located at the sixth or seventh node.

For all experiments the plants were grown under conditions of long photoperiod until they were ready for treatment. These photoperiods consisted of all the natural light available at the season the plants were growing plus approximately 40 foot-candles of incandescent-filament radiation from sundown to 2:00 A.M. The photoperiods thus exceeded 18 hours in the winter and 20 or more hours in the summer. This procedure has always been found to prevent floral initiation of Biloxi soybean and cocklebur, as determined by dissections made of representative plants at the time of the experiments. The experimental lots of plants were selected and moved from the greenhouse into the controlled-environment rooms described by PARKER (14). Here the soybeans were subjected 6 days, and the cocklebur 3 days, to short photoperiods using the methods described in detail below. During these experimental periods the plants differentiated a few new structures and made a small amount of growth but not enough to permit final determination of the effects of the treatment, so it was necessary to allow a few more days for their further development before the stem terminals were dissected. During this period the plants were again held in the greenhouse on long days. Dissections were made of all soybeans 14 days, and of all cocklebur 10 days, after the beginning of the experimental treatment.

Use of the growth chambers assured constant growing conditions during the experimental periods. Light intensities in the greenhouse both before and after the experimental treatments were subject to natural fluctuations, but, in general, these differences were not found to interfere with the results except during periods of unusually dark weather. At such times the plants were held on long photoperiods in the growth chambers for a few days prior to and following treatment.

During the experimental period the soybeans received 10-hour, and the cocklebur 12-hour, photoperiods of carbon-arc plus incandescent-filament radiation having a combined intensity at the

leaf surface of about 2000 foot-candles. Treatment of soybean and cocklebur in these growth chambers with the respective lengths and numbers of photoperiods indicated above invariably resulted in the production of flower primordia if the dark periods were uninterrupted. Floral initiation could be completely inhibited, however, if the dark periods were interrupted near the middle by brief periods of illumination of sufficient energy. It was thus possible through the application of a range of levels of energy in the form of dark-period interruptions to determine the threshold amount of energy that would just prevent flowering. These interruptions were made with filtered or white light in the reciprocity experiments and with relatively narrow wave-length bands of light from the spectrograph in all the action spectrum experiments. The energy levels required were such that, with the light intensities available, the duration of interruption of the dark periods ranged from about 1 to 25 minutes, except in a few experiments concerned primarily with the blue-violet end of the spectrum, in which interruptions as long as 40 minutes were necessary to inhibit floral initiation. In most experiments five different energy levels were used at each wave-length band tested. The sum of these five different periods of interruption was usually less than 1 hour, so that it was possible to complete all the interruptions in duplicate within a 2-hour period beginning 30 minutes before and ending 90 minutes after the middle of the dark period. This procedure, repeated in a second growth chamber operating on a cycle 2 hours later than the first, increased the number of replications of the entire experiment to four. In an average experiment fourteen or more bands of the spectrum were investigated in quadruplicate at five energy levels. The total number of plants involved in such experiments was 280 or more, exclusive of the controls which received short-day treatments but no dark-period interruptions. The individual lots of plants were brought from the control rooms and with the aid of a blue-violet safe-light, described below, the leaflets were placed in their respective positions on a board located in the image plane of the spectrograph.

The experimental results for soybean are expressed as total nodes at which flower primordia were formed and for cocklebur as total plants forming flower primordia. In soybean evidence for the effectiveness of a given treatment with short photoperiod is found in the production of flower primordia in the axil of the leaf situated several nodes below the terminal on the main axis (**2**). If the flower-forming stimulus is relatively weak, flower primordia may be formed only at this one node; but if the stimulus is stronger, they may be formed at one or more additional nodes above. The number of nodes on the main axis at which flower primordia occur is thus a semiquantitative measure of the effectiveness of the treatment. These values rather than the number of plants having flower buds are thus presented for soybean. Only when the stimulus is very strong and applied for a prolonged period does the terminal meristem give rise to a terminal inflorescence. In none of these experiments, however, was the treatment of sufficient intensity and duration to result in such a response.

In cocklebur, on the other hand, an effective treatment with short photoperiod results in the initiation of a terminal inflorescence. The only obvious difference in behavior of cocklebur plants that have received different intensities of photoperiodic stimulation is in rate of de-

velopment of the inflorescences. These differences can be expressed only by assigning the plants to various categories on the basis of size of the terminal inflorescences. This is unsatisfactory because of the possible influence of other factors on their rate of growth. For this reason the only record taken for cocklebur was the presence or absence of a recognizable inflorescence primordium, regardless of size.

Spectrograph methods and apparatus

Experiments designed to give the action spectrum for photoperiodic response required use of radiation of high intensity in a restricted wave-length region over an area somewhat larger than a leaflet of soybean or leaf of cocklebur. The wavelength band over a particular leaf had to be as narrow as possible, consistent with supplying energy adequate to suppress floral initiation as a result of exposures of less than an hour even in the least effective portions of the visible spectrum. This was achieved by means of the spectrograph described in the following paragraphs. Other experiments, performed to test the validity of the procedure used with the spectrograph, required only incandescent-filament lamps with or without filters.

The optical path in the spectrograph is shown in figure 1. Optical elements were a 25-cm. front-aluminized spherical concave mirror having a focal length of 2 meters, and two equilateral 60° flint glass prisms, with 14-cm. faces and 16.3 cm. in height. The slit and the focal plane were at the respective foci of the spherical mirror, thus giving a convergent beam through the prisms. The convergence leads to a loss of resolving power and thereby of spectral purity, which, however, is far within the limits required by the various experiments. The prisms were set at minimum deviation for the mercury green line. Light passing through the prisms was reflected from a front-aluminized flat mirror which was mounted so that it could be rotated about an axis in the mirror surface. Rotation was effected by means of a tangent screw on a 60-cm. arm. By turning the flat mirror, a given wave length of radiation could be brought to a specific position in the image plane of the spherical mirror. The image plane in most of the experiments was about 14 meters from the spherical mirror, which was adjustable in position to provide for focusing at any given position. The instrument and the light source, external views of which are given in figures 2*A* and 2*B*, were housed in a light-tight room having a length of about 18 meters.

Baffles were placed in the spectrograph to trap scattered light. Scattered light within the spectrum in the focal plane of the spherical mirror was reduced to an exceedingly low value, less than 0.1%, by the great distance from the last scattering surface, namely, that of the flat mirror. Constancy of light intensity throughout the course of an experiment was monitored with a light meter, having a barrier-layer type of photocell, placed in a portion of the spectrum from the edge of the first prism.

The slit of the spectrograph was 1.2 cm. high and could be adjusted to give widths as great as 1.5 cm. It essentially acted as a diaphragm on the projected image of the positive crater of the carbon arc which was used as a light source in most of the experiments. The carbon arc[5] was of the rotating cathode type using $\frac{9}{16}$-inch cored positive carbons. It could be operated at 12 kw. power input, and

[5] Supplied through the courtesy of Warner Brothers Theatres.

after several minutes' burning the light intensity at the monitor remained constant to within 3%. Under such operation a carbon lasted about 1 hour, and, although the arc would operate without attention for periods of 10–20 minutes, it was checked much more frequently to avoid deviations in energy output. Projection bulbs were used as light sources in some of the experiments with the red portion of the spectrum.

An adjustable slit that could be placed in the image plane of the spherical mirror served to define the spectrum for energy and wave-length calibration. The radiation passing this slit was focused on the receiver of a 10-junction thermopile, the response of which was calibrated with the aid of a standard lamp placed 6 meters from the slit. Wave-length calibration was carried out with the spectrograph slit illuminated by a low-pressure mercury arc. The spectrum position during the course of an experiment was checked against the absorption bands of a Didymium glass filter (Corning no. 512).

The dispersion of the spectrograph at 5000 A. is 15 A. per cm. At this dispersion the radiant intensity at 5000 A. can be as great as 3000 ergs per sq. cm. per sec. for an effective slit width of 100 A. In some experiments the distance to the image plane was shortened by moving the spherical mirror toward the slit, thereby decreasing the dispersion, in order to double the energy incident on a leaf in a given time. The spectrum for the greater image distance, which was generally used, measured about 2 meters over the visible region (4000–7000 A.) and had a height of about 10 cm.

Stations upon which leaflets were placed during irradiation were established on a board set in the image plane, as shown in figure 3*A*. The position of the spectrum was fixed with reference to a particular station in a given experiment. In all experiments with wave-length bands below 5500 A.—i.e., in the violet, blue, and green—leaflets were placed flat on the board and thus normal to the incident light beam (fig. 3*A*). A single leaflet was restricted to 10 cm. of the spectrum and subtended about 50 A. at 4000 A. and 150 A. at 5000 A. At the highest intensities an individual station thus covered a spectral region approximately one and one-half times the effective slit width.

In the region of the spectrum above 5500 A. dispersion decreased to such an extent that a single leaflet placed flat would have subtended about 200 A. in the region of 6000 A. Since use of such a

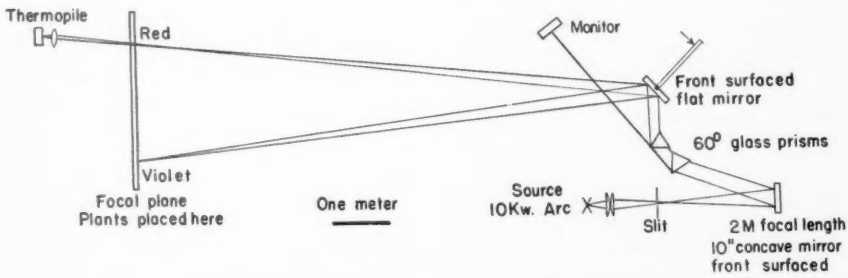

FIG. 1.—Diagram of optical path in spectrograph used for irradiation of plant leaves

wide band was objectionable, the leaflets for most experiments in this region were held on wedges at an angle of 30° to the light beam, as shown in figure 3*B*. The leaflets thus intercepted bands of only half the previous width or about 75 A. at 5500 A. and 100 A. at 6000 A. The purity of the spectrum in this region was increased by decreasing the slit width. The consequent reduction in energy was not objectionable because lower energies in this region of the spectrum were still effective.

Another procedure in a few experiments consisted in use of a lens arrangement that spread a small wave band of the red over a large area in which leaflets could be placed normal to the beam. A lens of 20-cm. focal length was mounted behind a slit in the image plane of the

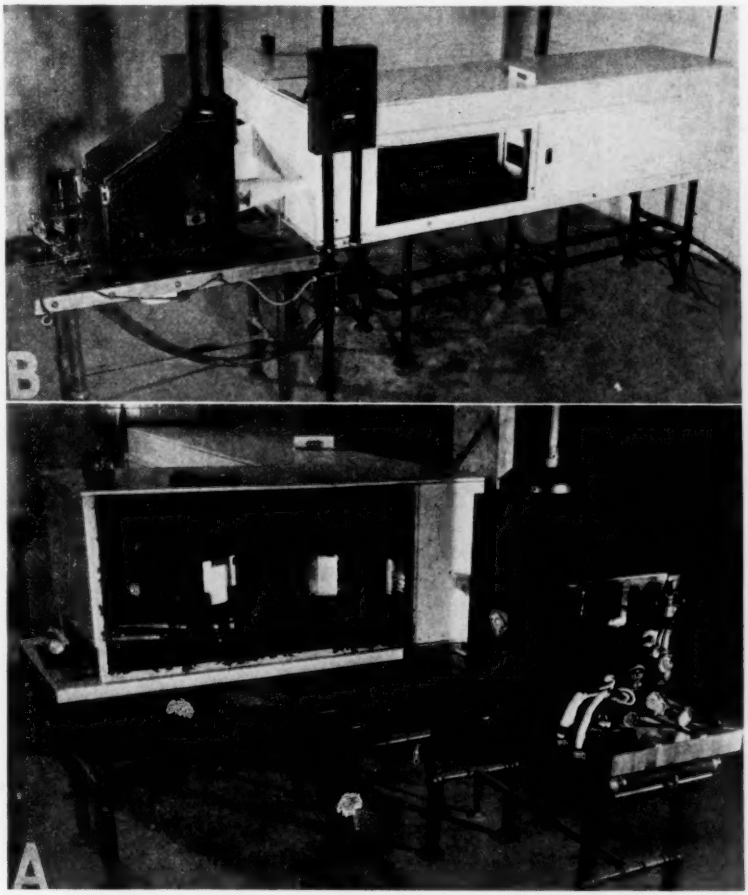

FIG. 2.—Views of spectrograph. *A*, front view with panel removed, showing two 60° flint-glass prisms and front-aluminized flat mirror attached to tangent screw. *B*, side view, showing light beam after passing slit located in cone attached to front of arc lamp.

spectrograph upon which a portion of the red end of the spectrum was incident. The intensity at the surface of the leaflets was dependent upon their distance from the lens and could be so adjusted that the exposure times could be increased to the same order as those in the blue. This arrangement also permitted direct comparison of the effectiveness of radiation at the two ends of the spectrum with the leaves placed normal to the incident beam in both cases.

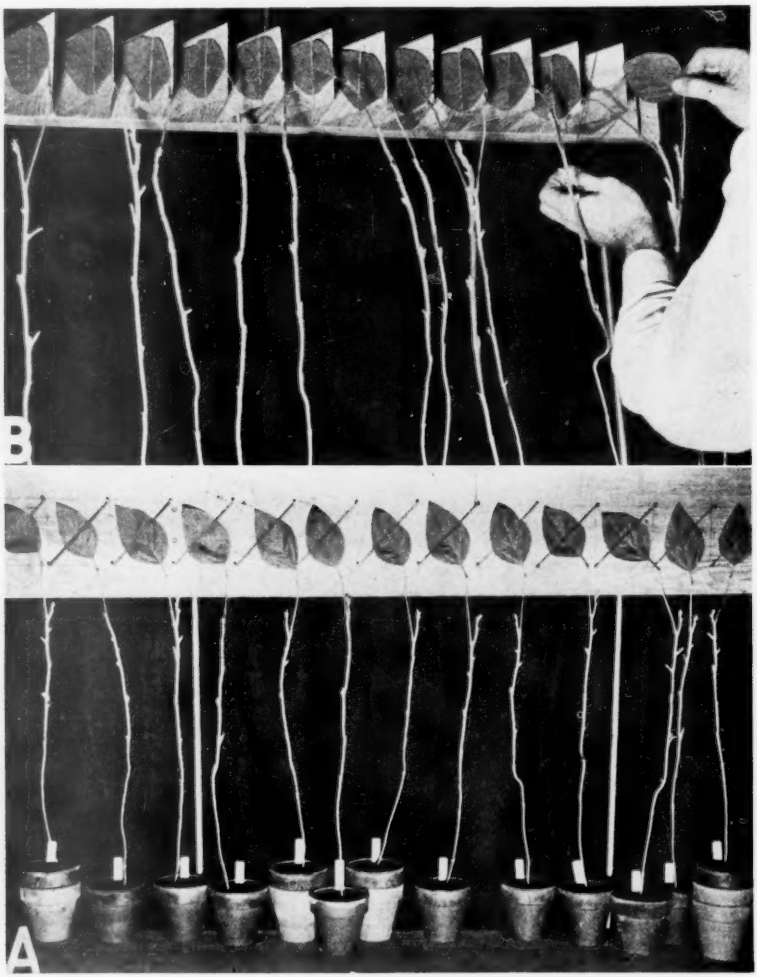

FIG. 3.—Methods employed to hold leaflets in image plane. *A*, setup of soybean leaflets held flat in image plane. *B*, setup of soybean leaflets held on wedges at angle of 30° to light beam. This type of setup used in orange-red region of spectrum and flat type was used in blue-violet region.

Experimentation and results

Reciprocity relationships, overnight threshold intensities, effect of leaf positions, and irradiation at various times after the start of the dark period

In experiments with the spectrograph different energies of irradiation for a particular wave-length region were given by varying time of exposure at constant intensity rather than by varying the intensity. With either time or intensity varying, it was necessary to test the reciprocity law, which states that the product of the duration and the intensity of light that will produce a certain effect is a constant. The validity of the spectrograph procedure required that the inhibiting effects on flowering caused by equal energies produced by such variations be constant within reasonable limits; that is, the procedure required that the reciprocity law hold for the factor of time and intensity over a range of approximately 1–60 minutes.

The reciprocity law was tested with both soybeans and cockleburs. The plants in all cases were defoliated in the manner previously described for the spectrograph experiments. They also received the same photoperiodic treatment; that is, the soybeans received six cycles of 10 hours of light and 14 hours of dark, and the cockleburs, three cycles of 12 hours of light and 12 hours of dark. The dark-period interruptions were near the middle in each case.

In any given experiment a single light source was used for the dark-period interruptions, and the desired intensities at the leaf surface were obtained by placing the plants at appropriate distances from it. The light source could have been any type that gave visible radiation in an effective region. In one experiment with Biloxi soybeans (table 1), an incandescent-filament lamp was used with a no. 244 Corning H.R. Lantern-red filter of standard $\frac{1}{4}$-inch thickness. Three groups of seven lots of ten plants each were included in the experiment. These groups received dark-period interruptions of 2, 6, and 18 minutes, respectively. The seven lots of each group were subjected to intensities such that corresponding lots of each group received the same total energies and successive lots within groups received progressively greater energies. The experiment included one lot of ten control plants which received no dark-period interruptions. With increasing energy in each group there was generally a decrease in number of flower primordia, the greatest changes occurring between the first and second and

TABLE 1

BILOXI SOYBEAN RECIPROCITY EXPERIMENT IN WHICH INTENSITIES WERE ADJUSTED SO THAT SEVERAL DIFFERENT QUANTITIES OF ENERGY WERE EACH APPLIED IN THREE DIFFERENT DURATIONS OF TREATMENT. RED LIGHT FROM A NO. 244 CORNING GLASS FILTER IN FRONT OF A 300-WATT INCANDESCENT-FILAMENT LAMP USED FOR DARK-PERIOD INTERRUPTIONS

Lot no.	Relative energy	No. of flower primordia formed on ten plants following different durations of dark-period interruption*		
		2 min.	6 min.	18 min.
1	1.00	24	23	14
2	1.67	16	8	14
3	2.34	6	4	6
4	3.00	7	0	4
5	3.64	2	1	0
6	4.31	0	0	0
7	5.00	0	0	0

* Controls had thirty-two flower primordia on ten plants.

the second and third levels, but floral initiation in corresponding lots of the three groups was about the same at corresponding energy levels. This indicates that, within the limits of time and intensity tested in this experiment, the reciprocity law held as closely as could be expected from the type of measurements performed with such biological material.

Reciprocity experiments on Biloxi soybeans similar to the above were also carried out with filtered radiation from a 600-watt G.E., a.c. Uviarc. The radiation was chiefly restricted to the mercury lines at 5461, 4358, and 4047 A. Energies required to suppress initiation of flower primordia were equivalent within a factor of two for exposures of 5, 12, 15, and 30 minutes. These times encompass the ones later used in determining the action curve for the violet and blue portions of the spectrum.

A reciprocity experiment with cocklebur was conducted in which the source of radiation for the dark-period interruptions was an unfiltered incandescent-filament lamp. The intensities of radiation were so adjusted that duration of interruption could be varied from 30 seconds to 64 minutes at seven constant-energy levels that differed from one another by a factor of slightly less than 1.5. The six plants of each experimental lot were subjected to dark-period interruptions within a period starting an hour before and ending an hour after the middle of the dark period; that is, during the sixth and seventh hours after the start of the 12-hour dark period.

Floral initiation was prevented in half or more of the plants of all but three lots at relative energy levels of 4.0 or higher (table 2). In all lots that received lower relative energies, on the contrary, two-thirds or more of the plants initiated flowers. Thus, the energies required to suppress floral initiation in the various groups were constant within the factor of 1.5 used between successive lots of a particular group.

Determinations were made for soybeans of the minimum intensity of illumination necessary to suppress floral initiation when given throughout the entire 14-hour dark period. In these experiments lots of plants were placed at various distances from a low-wattage incandescent-filament lamp in the same way as for one irradiation time of a reciprocity experiment. Three experiments, in each of which several lots of ten plants were used, gave the threshold intensity as 0.01, 0.02, and 0.02 foot-candles, respectively. The corresponding energies received by the leaflets of the plants were the same, within a factor of 3, as those required to prevent floral initiation when they were given in short photoperiods near the middle of the dark period.

The overnight threshold intensities for suppression of initiation of flower primordia by soybeans and cockleburs were directly compared. The soybeans were illuminated with unfiltered incandescent radiation for each of 6 nights of 14 hours as usual, and the cockleburs in two lots for 3 and for 6 nights of 12 hours each. The threshold intensity for the soybeans in this experiment was 0.01 foot-candles; that for the first lot of cockleburs was 0.01 foot-candles and for the second 0.02 foot-candles. Thus the two species, when compared under these conditions, required about the same intensity of continuous illumination to prevent floral initiation.

Overnight experiments were also carried out with violet light obtained by filtering the radiation from a 300-watt incandescent lamp through a no. 511 Corning Lantern-blue filter of $\frac{1}{4}$-inch thickness. Fifteen soybean plants placed 2 meters

from the light produced thirty-one flower primordia while fifteen plants held in the dark for the 14-hour nights produced forty. These results indicated that such a light had little if any effect in inhibiting floral initiation and that it could therefore be used as a safe-light during the setting-up of plants for the various experiments. To provide a still greater factor of safety, a 100-watt incandescent lamp was substituted for the 300-watt one. This lamp gave illumination sufficient for necessary manipulations and identifications of plants.

Leaflets of soybean plants in the reciprocity and threshold experiments were placed so that the surfaces were oriented between normal and 45° to the light beam. In the experiments with the spectrograph, leaflet surfaces were placed normal or at an angle of 30° to the light beam. Two experiments were conducted to test the relative effectiveness of irradiation under these various conditions. When the leaflets were placed at 30°, about twice as much energy was required as when the leaflets were placed normal to the light beam.

Since in the spectrograph experiments several lots were treated during a 2-hour period, it was necessary to know whether or not the energy required to prevent floral initiation was constant throughout this period. To obtain data on this point, a succession of threshold experiments was performed in which each experiment was started at a different time during the dark period. The intensity ranges were so adjusted that durations of interruption were 2–4½ minutes for soybean and 4–9 minutes for cocklebur. The energy required to prevent floral initiation in Biloxi soybean (table 3) was constant over the period from 1 hour before until 2 hours after the middle of the dark period. Results of two other similar tests indicated that about one and a half times more energy was required to suppress formation of flower primordia if applied at the end of 6 hours after the beginning of the dark period than if applied at the end of 7 or 8 hours. In view of these results all the dark-period interruptions for the spectrograph on soybeans were performed within a 2-hour interval starting

TABLE 2

COCKLEBUR RECIPROCITY EXPERIMENT IN WHICH INTENSITIES WERE ADJUSTED SO THAT SEVERAL DIFFERENT QUANTITIES OF RADIANT ENERGY WERE EACH APPLIED IN EIGHT DIFFERENT DURATIONS OF TREATMENT. WHITE LIGHT FROM INCANDESCENT-FILAMENT BULB USED AS LIGHT SOURCE

Lot no.	Relative energy	No. of plants per lot of six that formed flower primordia following different durations of dark-period interruption*								
		30 sec.	1 min.	2 min.	4 min.	8 min.†	8 min.†	16 min.	32 min.	64 min.
1	1.00	5	6	6	6	6	6	6	6	6
2	1.41	6	6	6	5	6	4	6	6	6
3	2.00	6	6	6	6	4	4	6	5	6
4	2.83	6	6	6	4	4	6	5	4	4
5	4.00	4	0	0	4	4	0	1	1	2
6	5.66	2	3	0	0	0	0	1	1	0
7	8.00	.....	2	0	1	2	0	0	0	0

* All uninterrupted controls flowered.

† Light source for first five exposure times differed from that of the last four. An 8-minute lot was run with both sources.

30 minutes before the middle of the 14-hour dark period.

Similar experiments conducted for cocklebur (table 4) gave essentially the same results. Results obtained during an interval of $2\frac{1}{4}$ hours beginning at the middle of the dark period were practically constant. This was verified in four other experiments. The spectrograph experiments were restricted to the 2-hour period immediately following the middle of the 12-hour dark period.

A striking feature of these experiments was the asymmetry in plant response with respect to the middle of the dark period. Results presented in tables 3 and

TABLE 3

EFFECT ON BILOXI SOYBEAN OF IRRADIATION WITH WHITE LIGHT AT SEVERAL ENERGY LEVELS AND AT VARIOUS TIMES AFTER START OF 14-HOUR DARK PERIOD
DURATIONS OF IRRADIATION RANGED FROM 4 TO 9 MINUTES

Relative energy	No. of primordia formed per lot of eight plants following irradiation after various numbers of hours from start of dark period										
	4.0	4.5	5.0	6.0	7.0	8.0	9.0	10.0	10.5	11.0	11.5
1.00	.....	.....	.....	.....	9	13	13	17	.....	.....	.....
1.50	.....	.....	.....	6	9	10	9	13	15	.....	.....
2.25	.....	.....	.....	4	10	9	7	15	15	.....	.....
3.40	.....	.....	.....	2	2	1	1	8	10	13	.....
5.10	4	5	6	0	1	0	0	4	2	9	10
7.60	8	5	2	0	0	0	0	1	1	7	9
11.40	7	7	1	0	0	0	0	0	0	1	11
17.00	2	0	0	0	.....	.....	.....	.....	0	0	4
25.60	1	0	0	.....	.....	.....	.....	.....	.....	0	9
38.40	0	0	0	.....	.....	.....	.....	.....	.....	0	2
57.60	0	0	0	.....	.....	.....	.....	.....	.....	.....	4
Controls*	7				16	12		22			

* Experiment conducted with plants in four different control rooms. Each group to be compared with its own control.

TABLE 4

EFFECT ON COCKLEBUR OF IRRADIATION WITH WHITE LIGHT AT SEVERAL ENERGY LEVELS AND AT VARIOUS TIMES AFTER START OF 12-HOUR DARK PERIOD
DURATIONS OF IRRADIATION RANGED FROM 2 TO $4\frac{1}{2}$ MINUTES

Relative energy	No. of plants per lot of eight forming flower primordia following irradiation after various numbers of hours from start of dark period*							
	3.75	4.50	5.25	6.00	6.75	7.50	8.25	9.00
1.00	.....	8	8	8	8	8	.....	.....
1.50	8	8	8	8	8	8	8	.....
2.25	8	8	8	8	8	8	6	8
3.40	8	8	8	4	2	2	5	5
5.10	8	7	4	2	1	1	3	5
7.60	7	4	2	1	1	0	1	4
11.40	4	3	0	1	0	0	2	3
17.00	3	.....	.....	.....	.....	.....	1	1
25.60	.....	.....	.....	.....	.....	.....	.....	1

* All controls on short day flowered.

4 are representative of others obtained. They show, for instance, that at least three times as much energy was required to suppress floral initiation of cocklebur when given 1.5 hours before the middle of the dark period as contrasted with 1.5 hours after the middle. Soybeans exhibited a similar difference in sensitivity to treatments at 3 hours on either side of the middle of the dark period. In general, the asymmetry for both plants was such that markedly greater energy was required to suppress floral initiation when the radiation was given appreciably before rather than after the middle of the dark period.

If the time of interruption was so placed as to permit a portion of the dark period to approximate that required for flowering, the response was variable. This for soybean (table 3) was illustrated by the strikingly different behavior following interruption at 11 or at 11½ hours after the start of the dark period. Similar results were obtained in experiments undertaken to determine the critical day-length necessary for flowering of cocklebur.

Limits of effectiveness in the spectrum

While spectrograph experiments were performed with high energies at wave lengths as great as 9000 A., it was still considered necessary to test further for possible effectiveness of infrared radiation in preventing floral initiation. This was done by irradiating fifteen soybean plants throughout the night at 1.2 meters from a 300-watt lamp behind a Corning no. 254 filter of ¼-inch thickness which did not transmit wave lengths shorter than 7500 A. These were compared with fifteen other plants that received no dark-period interruptions. All plants of each lot formed flower primordia, and there was no difference between lots in the number of primordia formed. Only about 10 seconds' irradiation in the region of 6500 A. under these conditions would have been required to prevent initiation of primordia.

Spectrograph experiments were not completely satisfactory in establishing the nature of the photoperiodic response to radiation in the near ultraviolet. This was caused by a probable lower or possible zero effectiveness of such radiation, by the steadily decreasing intensity in the radiation source at shorter wave lengths, by the increased dispersion of the spectrograph, and by the decrease in transmission of the prisms. For these reasons tests were made with the 3650 A. mercury line isolated by means of filters. The radiation source was a 600-watt G.E., a.c. Uviarc equipped with no. 586 and no. 738 Corning filters, each of ¼-inch thickness. Ten soybean plants exposed for 1 hour at 1.0 meter from this source initiated twenty flower primordia, while ten control plants that received no dark-period interruption initiated twenty-four primordia. Thus at 3650 A., energies as great as 2000 kiloergs per cm.^{2} did not inhibit the formation of flower primordia, whereas lower energies were inhibitory in even the least effective region of the visible spectrum.

Action spectrum for prevention of floral initiation by Biloxi soybeans

After a number of preliminary tests, fourteen spectrograph experiments were performed to determine energies required at various wave lengths to suppress initiation of flower primordia by Biloxi soybeans. Six of these were designed primarily for the violet-blue portion of the spectrum, but they included a considerably wider region. The maximum

time of exposure was 21 minutes, and each exposure was repeated on four lots of plants.

In one experiment in this region (fig. 4), 220 plants were irradiated at fourteen different wave-length regions and for six them between points at which sharp breaks occurred in the numbers of flower primordia produced. The maximum energy required to prevent floral initiation in this region was about 1200 kiloergs at 4800 A. Below this wave length the re-

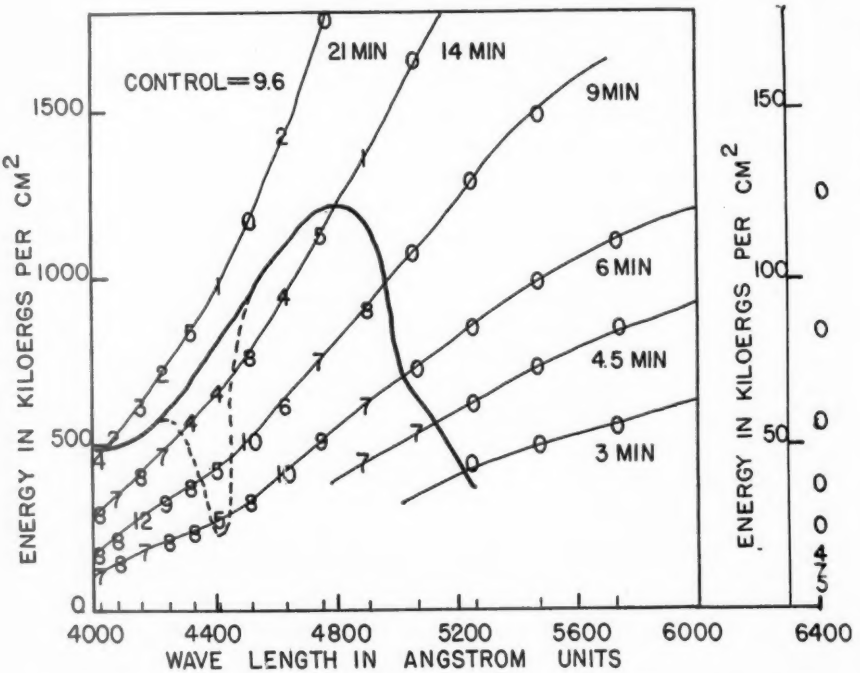

FIG. 4.—Response curve for suppression of floral initiation in Biloxi soybean in blue-violet region of spectrum. Leaflets were held at fourteen different wave-length stations for six different durations. Resulting energy at each wave-length station is plotted, and superimposed on energy lines are total numbers of flower primordia formed by lots of four plants which received a specific wave-length band at different energy levels. Response curve was drawn freehand through energy lines, crossing them between points at which sharp breaks occurred in number of flower primordia produced. Dotted portion of curve at 4400 A. suggests the possibility of an enhanced effect in this region. Results of an experiment to compare effectiveness of radiation at 6400 A. with that in blue-violet region are shown at extreme right of figure.

different durations. The resulting energies at each wave-length station are plotted on the graph. Superimposed on these energy lines are the total numbers of flower primordia formed by lots of four plants which received a specific wave-length band at different energy levels. A response curve was drawn freehand through the energy lines, crossing sponse curve approximately followed the radiation curve (i.e., 21-minute exposures were more effective at all wave lengths than 14-minute exposures). Thirty-six plants were maintained as short-day controls. These developed an average of 2.4 flower primordia each, or 9.6 for a group of four plants. The response curve of a second experiment

(fig. 5), extending to slightly shorter wave lengths, had the same general features of that of figure 4. In both these experiments and in one previously described (15), radiation near 4400 A. was perhaps somewhat more effective in preventing floral initiation than was that of neighboring regions. No evident effect of the type suggested by the dotted line of figure 4 was observed in the other experiments in the violet-blue portion of the spectrum. If such an effect occurs in this region, it is close to the limit of detection imposed by the method of measurement and the variability of the biological material.

Response curves taken from the six experiments in which the violet-blue portion of the spectrum was used (fig. 6) show approximately a twofold variation between the extreme curves. The relative response in the various wave-length regions for a particular experiment was, of course, far less variable; that is, the shape of a response curve was determined very accurately within each experiment, but the absolute value of the ordinates varied somewhat from one experiment to another. This variability between experiments was the result of the variable environmental conditions under which the plants were produced in the green-

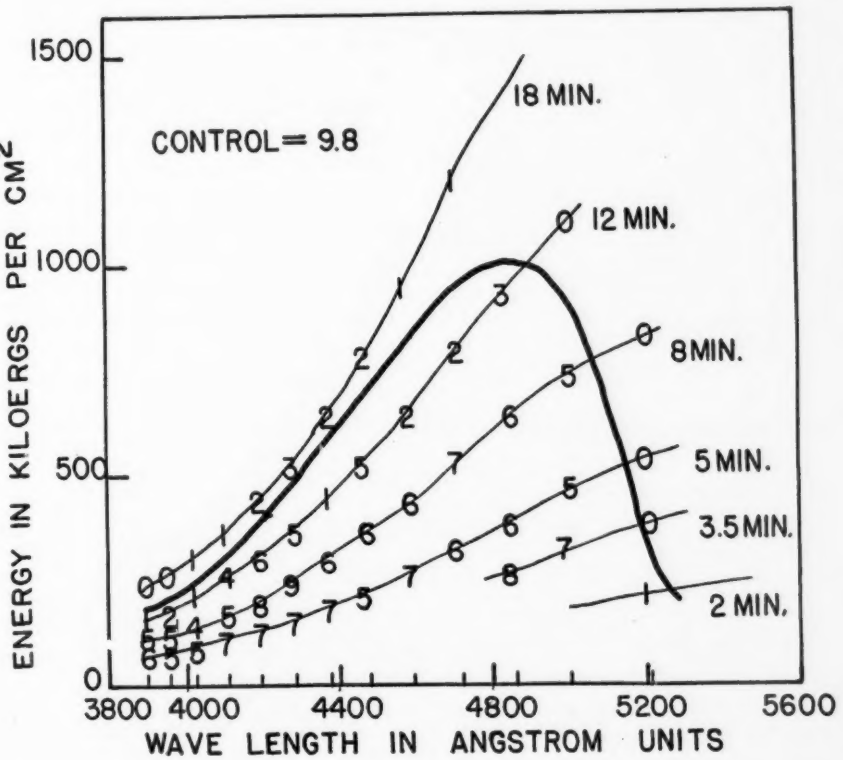

FIG. 5.—Another response curve for suppression of floral initiation in Biloxi soybean in blue-violet region of spectrum.

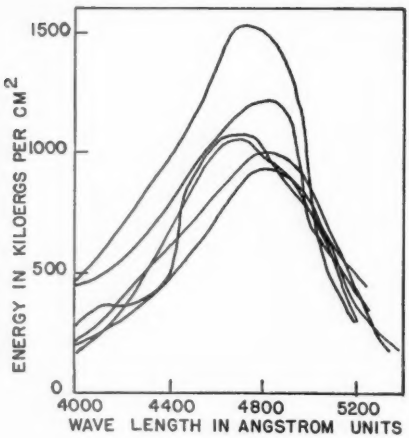

FIG. 6.—Response curves for suppression of floral initiation in six different experiments with Biloxi soybean in blue-violet region. There is approximately a twofold variation between extreme curves.

house, since experiments were performed at all seasons of the year.

Seven experiments were performed in the region between 5400 A. and 7800 A. For these experiments the leaves were held against the wedges at an angle of 30° to the light beam, as previously described.

In one experiment (fig. 7) the range from 5500 A. to 6800 A. was covered by stations the centers of which were 5.5 cm. apart. The range from 6800 A. to 7900 A. was covered by five stations that were 3.75 cm. apart. A narrow slit was used in the spectrograph in order that the effective slit width could be maintained at about one-half the width of the leaf projected along the light beam. The solid curve (fig. 7) was taken as the one representing the plant response for com-

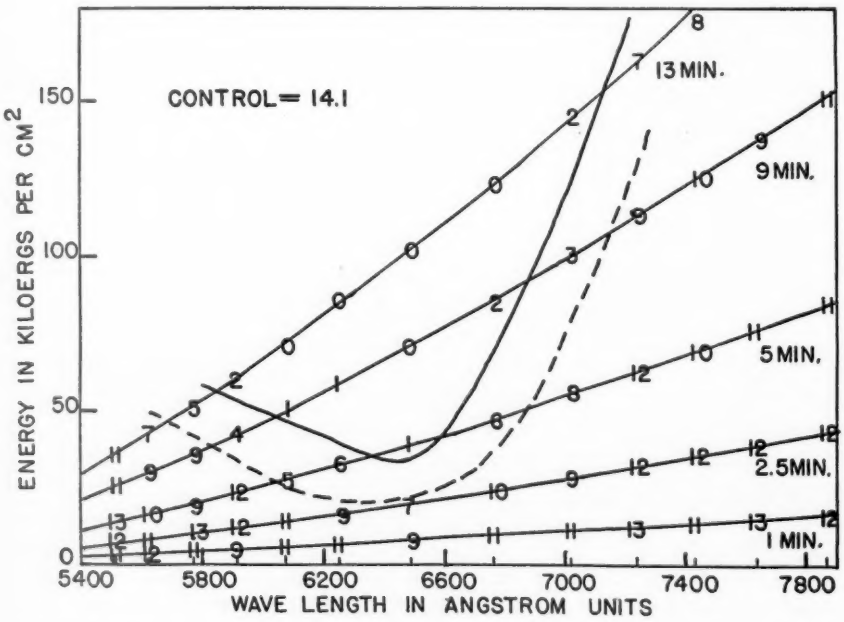

FIG. 7.—Response curve for suppression of floral initiation in Biloxi soybean in range from 5500 A. to 7900 A. Solid curve was taken as one representing the response, while broken-line curve illustrates gradation effect with decreasing energy.

parison with the action curve in the violet-blue portion of the spectrum. It followed the energies required at the various stations for practically complete suppression of flower primordia. A dotted curve below this one illustrates a gradation effect of decreasing energy.

The long-wave-length limit of radiation effective in preventing floral initiation was established by four spectrograph experiments. One of these (fig. 8) was particularly designed to trace the response curve as far toward long wave lengths as was feasible. Approximately 2300 kiloergs per sq. cm. were required to suppress primordium development at 7300 A. However, some effect on floral initiation was possibly caused by high energies at 7700 A.

Response curves for seven separate experiments (fig. 9) in the yellow-red portion of the spectrum show very close agreement in the region for maximum

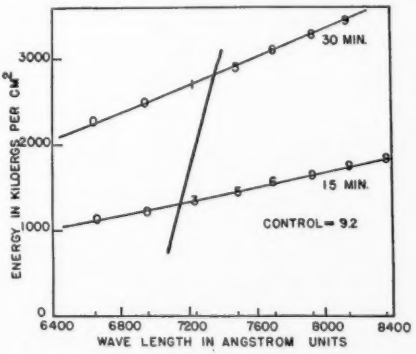

FIG. 8.—Results of one of the experiments to establish long-wave-length limit of radiation effective in preventing floral initiation.

effectiveness. One of these curves covered wave lengths down to about 5400 A. and served to tie this group of experiments to those of the violet-blue region of the spectrum which covered the range of approximately 4000 A. to 5400 A. The region covered by this particular curve was one over which the effectiveness of radiation was changing rapidly.

Energies required to suppress development of flower primordia of Biloxi soybean at the most effective regions in the

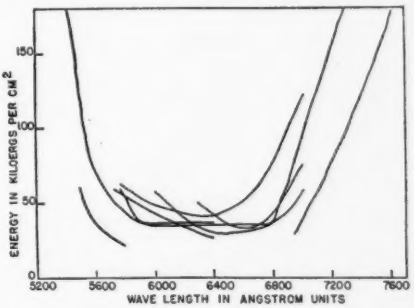

FIG. 9.—Response curves for seven separate experiments in yellow-red portion of spectrum.

red and the violet and at the least effective region in the blue portion of the spectrum are shown in table 5. The most direct comparison between these values was given by the experiments of 9–26–45 and 9–17–45, which were designed to compare effectiveness of radiation in a narrow region of the red with that in the blue-violet region of the spectrum. These experiments made use of the previously described lens arrangement at the red end of the spectrum centered at 6400 A., which permitted all leaves to be placed normal to the light beam and increased the radiation times in the red to the same order as those of the violet-blue. The data of the experiment of 9–26–45 (table 5) are shown with the results obtained at 6400 A. on an ordinate one-tenth that used for the rest of the data (fig. 4). In this experiment the energy required to prevent floral initiation at 4800 A., the least effective region in the visible, was about sixty times as great as the energy required at 6400 A.

TABLE 5

SUMMARY OF ENERGIES REQUIRED TO PREVENT FLORAL INITIATION IN BILOXI SOYBEAN IN MOST EFFECTIVE REGIONS OF RED AND VIOLET AND IN LEAST EFFECTIVE REGION OF BLUE

DATE	KILOERGS PER SQ. CM.			LEAF POSITION IN RELATION TO LIGHT BEAM
	Red	Blue	Violet	
9–26–45	20	1200	450	Normal
9–17–45	25	1500	500	Normal
7–10–44	40	.....	.....	30°
6– 5–44	27	.....	.....	30°
5–29–44	34	.....	.....	30°
5–22–44	36	.....	.....	30°
5– 8–44	24	.....	.....	30°
9– 4–44	.....	900	200	Normal
8–22–44	.....	1000	220	Normal
7–31–44	.....	1100	300	Normal
2–27–45	.....	1000	200	Normal

The data of all experiments with Biloxi soybeans were combined to give an action spectrum (fig. 10). The experiments of 9–26–45 and 9–17–45 (table 5) were very helpful in establishing a common ordinate for the two halves of the curve, a matter concerning which there had previously been a twofold factor of uncertainty (15).

ACTION SPECTRUM FOR PREVENTION OF FLORAL INITIATION BY XANTHIUM SACCHARATUM

The action spectrum for *Xanthium* was determined from the results of four experiments in the red portion of the spectrum and four in the blue. The detailed data presented for certain of those

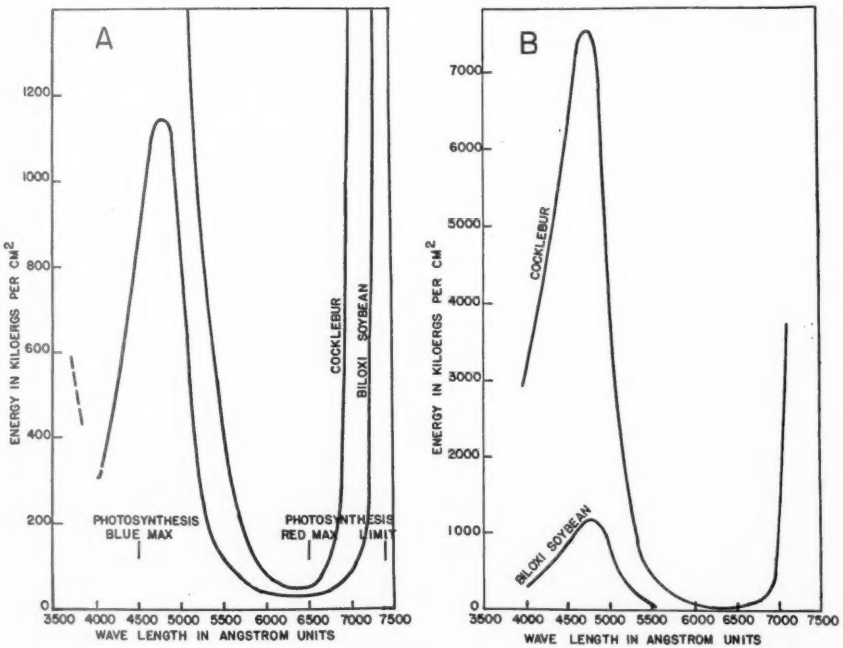

FIG. 10.—Composite action spectrum for suppression of floral initiation in soybean and cocklebur. *A*, points on soybean curve give energy required at middle of a 14-hour dark period to prevent floral initiation. Wave lengths for maximum photosynthesis and wave-length limit as measured by HOOVER (8) are shown. *B*, points on cocklebur curve give energy required at middle of a 12-hour dark period to prevent floral initiation.

experiments (figs. 11, 12, and 13) were expressed in terms of the number of plants per lot of four that initiated flower primordia following treatment.

The action curves (fig. 14) for the four experiments in the red region, based upon about 1200 plants, agreed with one another within reasonably narrow limits between 5400 A. and 6900 A. The broken-line curve approximates a mean for the four experiments, although it gives relatively little weight to the one response curve that shows lowest energy between 5500 and 6600 A.

Since the experiments with cocklebur in the red region were performed with the carbon-arc lamp, the times of exposure to produce a given amount of energy were very much shorter than those for

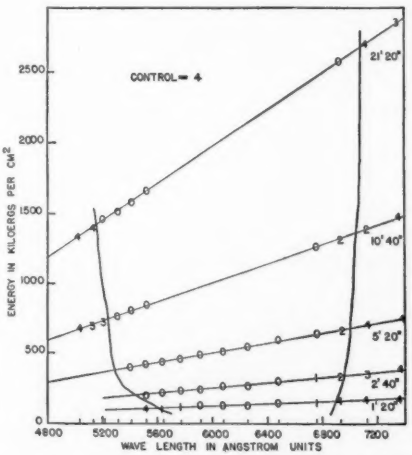

FIG. 11.—Response curve for prevention of floral initiation in cocklebur. Figures superimposed on energy lines indicate number of plants from each lot of four that formed flower primordia.

the soybean experiments performed with the projection lamp (fig. 7). One of these cocklebur experiments (figs. 11 and 12), which was performed with energies as great as 2500 kiloergs per sq. cm., showed that radiation at wave lengths longer than 7000 A. was very ineffective in preventing floral initiation in cocklebur. This limit thus occurred at a somewhat shorter wave length for cocklebur than

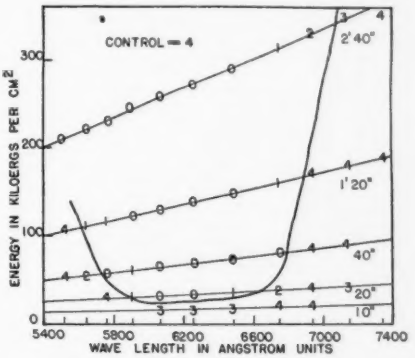

FIG. 12.—Continuation of lower part of curve shown in fig. 11. Region of maximum effectiveness for preventing floral initiation in cocklebur was about 6300 A.

for soybean. The region of maximum effectiveness for cocklebur was about 6300 A. and was somewhat more sharply defined than in the case of soybean.

Preliminary experiments indicated that it would be difficult to obtain adequate energy in the blue portion of the spectrum to permit tests equivalent to the ones used for soybeans. The energy in the specific wave-length regions was accordingly made as great as the limiting factors permitted by reducing the distance to the image plane, broadening the slit, running the arc at the maximum power input, and increasing the time of irradiation as much as was feasible. The resulting decrease in dispersion and resolving power was not sufficiently great to interfere with the results. Under the conditions employed floral initiation of cocklebur could be suppressed with exposures of 50 minutes in the blue-violet region from 3900 A. to 4800 A. and with

considerably shorter time at longer wave lengths. With such long exposures it was not possible to treat more than four plants per station during the 4-hour duration of an experiment. However, since

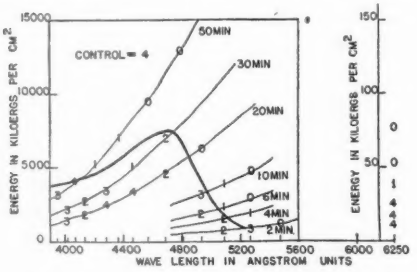

FIG. 13.—Response curve for prevention of floral initiation in cocklebur in blue-violet portion of spectrum. Data on extreme right show results of an experiment that was used to compare effectiveness in blue-violet region of spectrum with that in red region.

the dispersion was so great in the blue-violet region from 3900 A. to 4800 A., adequate sampling was attainable by confining the 50-minute treatments to five alternate stations. The intervening stations in this range and all stations above 4800 A. were used for the shorter exposures (fig. 13). The region of least effectiveness was near 4800 A., and this region was also found least effective in soybean, but the energies required to prevent floral initiation were far greater for cocklebur.

Response curves from the four experiments performed in the blue-violet region were very similar (fig. 15) and resulted from experiments performed with different lots of plants grown during the period of May–August, 1945. Any variability between these lots did not alter the general character of the curves for the action spectrum.

Direct comparison was made of the effectiveness of radiation in the blue-violet portion of the spectrum with that of the most effective part of the red in the same way as described for soybeans. These comparisons were made during the course of the four experiments in the blue region. The energy ratios between the least effective region of the blue and the most effective portion of the red (table 6) were 250:1, 150:1, 200:1, and 220:1 in the four experiments. These ratios were above three times greater than found for soybeans, owing chiefly to lower effectiveness of the blue radiation. Aside from this, the responses of soybeans and cocklebur were closely similar, as shown in the composite curve of the action spectrum (fig. 10*B*).

Discussion

Experiments reported were undertaken to gain information about the initial step in the prevention of flowering

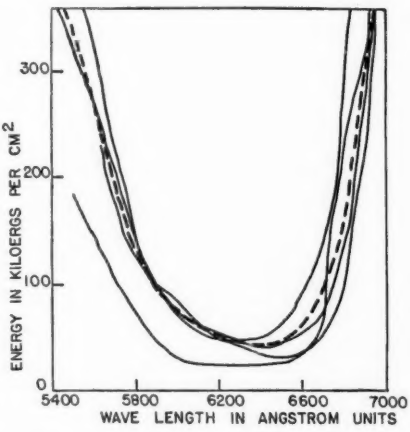

FIG. 14.—Response curves for cocklebur from four experiments in yellow-red region of spectrum are shown by solid curves. Broken-line curve shows average selected from this group.

in short-day plants by the action of light. Many workers (4, 12, and 13) have shown that the photoperiodic reaction occurs in the leaves and that it can be controlled by the action of visible

radiation. It follows, therefore, that some colored compound in the leaf is involved in the initial step of this photoreaction. This compound might be closely allied to the material that is transported to the meristem where it regulates initiation, or it might be one of the common leaf pigments which enters into photochemical reactions thereby preventing the appearance of a regulatory substance at the terminal meristem.

The simplest observation to be made from the data is that both Biloxi soybean and cocklebur have action curves that are similar in many respects. Both plants respond photoperiodically to radiation from all portions of the visible spectrum, but the energy required to prevent floral initiation in either plant varies greatly over the spectrum. Positions of minimum and maximum effectiveness in the spectrum and the long-wave-length limit of response are the same for both plants. This, at least, is an

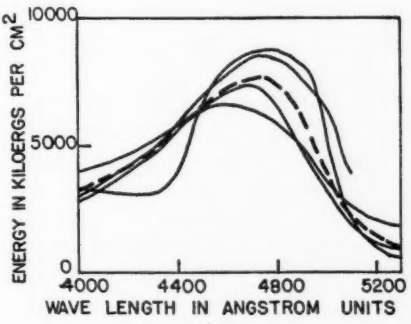

FIG. 15.—Individual action spectra for four experiments with cocklebur in blue-violet portion of spectrum are shown by solid curves. Selected average is shown by broken-line curve.

indication that the same pigment or pigments are involved in the photoperiodic response of the two plants.

The ideal case for studying a biological response to light is one in which the action curves can be corrected for scattered light and for absorption by screening pigments, the latter requiring a priori knowledge about the effective pigment. If possible, the system should not absorb too much of the incident energy. These conditions are not realized in measurement of the photosynthetic action curve for higher plants because of leaf structure but have been attained for unicellular green algae (**6**). In the case of photoperiodic control of flowering no method has yet been devised to avoid the complexities of light absorption in the leaves of the plants.

TABLE 6

SUMMARY OF ENERGIES REQUIRED TO PREVENT FLORAL INITIATION IN COCKLEBUR IN MOST EFFECTIVE REGIONS OF RED AND VIOLET AND IN LEAST EFFECTIVE REGION OF BLUE

DATE	ENERGY IN KILOERGS PER SQ. CM.			LEAF POSITION IN RELATION TO LIGHT BEAM
	Red	Blue	Violet	
12- 4-44	60	.....	.....	Normal
6- 6-45	50	.....	.....	30°
7- 3-45	25	.....	.....	30°
7-18-45	30	.....	.....	30°
8-21-45	30	7500	3500	Normal
7- 7-45	55	8500	2500	Normal
5-31-45	50	10000	3000	Normal
8- 7-45	30	6500	2500	Normal

The possibility that the action curve might be an expression of absorption by a common leaf pigment will be examined in detail. Pigments in question are carotinoids and chlorophylls *a* and *b*. The action spectra indicate effectiveness of light at wave lengths as long as 7000 A. in controlling floral initiation and thus definitely rule out the first class of compounds other than in their screening action. The features of the action curve that suggest that chlorophyll may be the effective pigment are the long-wave-

length limit, the general effectiveness throughout the visible, the most rapid change in response at the steepest portion of the chlorophyll-absorption curve, and the presence of two maxima of response, one in the red and the other in the violet. Many factors must be discussed in considering the possibility that chlorophyll may be the effective pigment, and, in the end, decision must remain for future work.

Familiar action curves involving chlorophyll as the effective pigment are those for photosynthesis. These curves have been determined for wheat by HOOVER (8) and for *Chlorella* suspensions by EMERSON and LEWIS (6). In the former case the amount of CO_2 fixation for unit incident energy varies but little across most of the visible spectrum, which is to be expected with great amounts of the absorbing pigment in the light path. In the latter investigation the oxygen evolution was shown to follow the absorption spectrum of chlorophyll with constant quantum efficiency. Expressed in another way, maximum photosynthesis for dilute *Chlorella* suspensions was obtained for unit incident energy at the absorption maxima and less in the more transparent regions (EMERSON and LEWIS, fig. 7). Both curves, however, have the same long-wave-length limit of response. This limit, moreover, is the long-wave-length limit of light absorption by isolated chloroplasts (5). It corresponds within the limit of experimental error to the long-wave-length limit for photoperiodic response of soybean and cocklebur, and this correspondence might be expected irrespective of the detailed nature of the two processes provided the red absorption bands are effective in both.

Photosynthetic effectiveness for wheat reaches a slight maximum near 4400 A. and decreases greatly between 4400 and 4000 A. The maximum effectiveness for stopping floral initiation apparently takes place at shorter wave lengths. About half the experiments with the violet-blue portion of the spectrum suggest an enhanced effect of radiation near 4400 A., as indicated by the dotted line on figure 4. The least effective portion of the spectrum is near 4800 A. for both soybean and cocklebur. The two species, however, differ significantly in the effectiveness of such radiation in comparison with the most sensitive region in the red. Ratios of energies required to stop floral initiation are:

Soybean E (4800 A.) : E (6400 A.) = 60 : 1
Cocklebur E (4800 A.) : E (6400 A.) = 200 : 1

This region of minimum photoperiodic effectiveness is near the wave lengths at which chlorophylls *a* and *b* have their maximum absorption coefficients.

If chlorophyll is the pigment responsible for the initial step of photoperiodic control of flowering, then the immediate reaction takes place in the chloroplasts, since absorbed energy can be transferred only a distance of a few molecular diameters. The absorbed energy effective in preventing flowering of short-day plants is probably transferred to some compound appearing in a chain of chemical reactions that do not depend upon absorbed light. In the absence of light a product of these reactions reaches sufficient concentration so that the amount diffusing away from the chloroplasts and finally reaching developing meristematic tissue is sufficient to control development. The compound to which the energy is transferred might thus undergo a side reaction, such as oxidation, which would break the chain of reactions lead-

ing to the final effective compound. The break could be at any point along the chain, including the final reaction.

If chlorophyll is the effective pigment, then possibly the chlorophyll-containing cells must absorb enough energy to reduce the concentration of a compound entering the photoreaction to some definite but unknown average level. Radiation in the region of maximum absorption will be more reduced in intensity at the chloroplasts farthest from the irradiated surface than will radiation having less absorption. If the energy received is below a certain value, these particular chloroplasts will still supply the active compound. In regions of low absorption of light by the chloroplasts, near 5600 A., for example, radiation is less reduced at the chloroplasts farthest from the irradiated surface, and the actual energy absorbed is possibly greater than for the region of the maximum absorption coefficient. As a result less energy could be required, as actually observed, for suppression of floral initiation at 5600 A. than at 4800 A., where the absorption coefficient is greater.

Change of light absorption with depth in an absorbing medium might be considered in greater detail to illustrate the effect. The intensity at a particular depth (x) is given by

$$I = I_0 e^{-ax},$$

where I_0 is the incident intensity and a is the absorption coefficient. Energy absorbed at a particular depth is given by the derivative of the above expression with respect to depth, or

$$\frac{dI}{dx} = -aI_0 e^{-ax}.$$

At the surface of the absorbing medium x is zero and dI/dx is $-aI_0$. Thus the energy absorbed at the surface for a given incident energy depends directly upon the absorption coefficient. As the depth increases ($x>0$), the absorption decreases, that is, e^{-ax} decreases with increasing values of x, but at a different rate for each absorption coefficient (a). The rate of change of absorption decreases as the absorption coefficient decreases, and a depth is finally reached at which absorption of strongly absorbed radiation becomes less than that of weakly absorbed radiation. This change of absorption with depth in a homogeneous medium is illustrated in figure 16 for three absorption coefficients corresponding to three different wave lengths of incident light having the same intensity I_0 at each wave length. At a depth of 2.5 units ($x = 2.5$) about ten times as much energy is absorbed per unit of volume for an absorption coefficient of 0.5 as for 2.0. At the surface, of course, the absorption was four times as great for the latter as for the former. In general, the effect of self-screening by the chloroplasts or within a chloroplast could be of the type to broaden the region of relative great sensitivity in the red, orange, and yellow, as contrasted with the absorption spectrum of chlorophyll, and to reduce the effectiveness of the blue.

Consideration of phototropic movement of plants is of interest in contrast to photoperiodic control of flowering. The action curve for the bending of the Avena coleoptile was first measured by BLAAUW (1) and later by JOHNSTON (9). Both observers found a maximum bending response in the neighborhood of 4400 A. with a second near 4750 A. Radiation beyond 5500 A. does not produce bending. WALD and DU BUY (17) indicated that the effective pigment is probably a carotinoid, and, by extracting oat coleoptiles, they obtained a solution having

an absorption spectrum similar to the action curve. The initial step is the absorption of light by the carotinoids of the plant, but control of bending is brought about through the action of auxins which

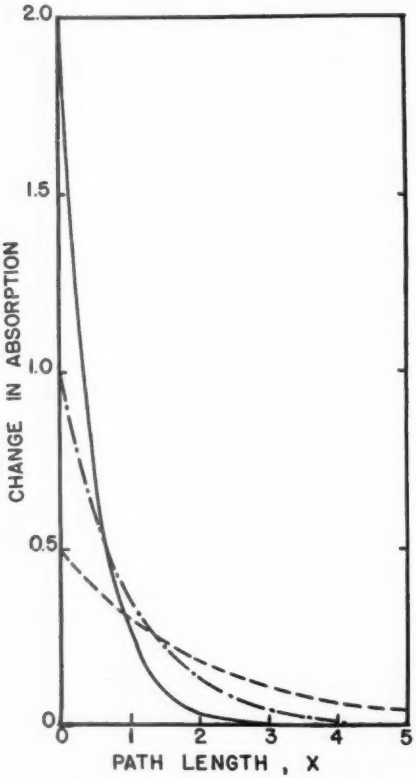

FIG. 16.—Change in energy absorbed with depth for three absorption coefficients corresponding to three different wave lengths of incident light:

$$\frac{dI}{dx}(\text{ordinates}) = -aI_0e^{-ax}.$$

are colorless compounds. A reaction chain of considerable length undoubtedly exists between the initial step and the final control.

Reciprocity experiments and experiments involving irradiation at various times after the start of the dark period, which were conducted to determine the practicability of certain experimental routines, also contribute significant data concerning the nature of the photoperiodic reactions involved. These experiments indicate that the compound involved in the photochemical reaction either never diffuses from the chloroplasts or diffuses very slowly.

Summary

1. The purposes of this investigation were to obtain quantitative data on the photoreactions that prevent flowering of short-day plants, from which an action spectrum relating wave length to photoperiodic effectiveness of light could be derived, and to draw such inferences concerning the nature of the photoreactions as the action spectrum would permit.

2. Experiments designed to give the action spectrum made use of a specially designed prism spectrograph having a dispersion of 15 A. per cm. at 5000 A. At this wave length and with an effective slit width of 100 A. the energy was about 3000 ergs per sq. cm. per second with the slit illuminated by a carbon arc operated at 12 kw. input.

3. Plants investigated were soybean, *Soja max* (L.) Piper var. Biloxi, and cocklebur, *Xanthium saccharatum* Wallr. To facilitate irradiation, the foliar surface of the plants of each species at the beginning of an experiment was reduced to a single leaflet or leaf, respectively.

4. The experimental treatment was based on the fact that flowering of short-day plants receiving short photoperiods can be prevented by interrupting each of the accompanying long dark periods at or near the middle with a brief period of irradiation. In these experiments minimal energies required to prevent floral initiation were determined for many narrow regions of the spectrum.

5. The reciprocity law was tested and found to hold for the factors of time and intensity within the ranges used for dark-period interruption. Flowering response of both soybean and cocklebur depends on the total radiant energy used to interrupt the dark period.

6. Minimal energies necessary to prevent flowering depend upon the number of hours of darkness elapsing before the plants are irradiated but are constant over a 2-hour period beginning 30 minutes before the middle of the dark period.

7. Action spectra for soybean and cocklebur are similar in several respects. The limit of effectiveness of radiation for preventing flowering of both plants is near 7200 A., at the red end of the spectrum. Maximum effectiveness for both plants occurs over a broad region extending from about 6000 to 6800 A. A minimum of effectiveness of radiation for both plants occurs in the region of 4800 A., and effectiveness increases again at shorter wave lengths in the visible portion of the spectrum.

8. Ratios of energies required to prevent flowering in the regions of maximum and of minimum effectiveness were different for the two plants, being about 1:200 for cocklebur and 1:60 for soybean.

9. The possibility that chlorophyll may be the effective pigment in the photoperiodic reaction is examined.

Mrs. LAURA E. RAPPLEYE and Miss EMILY DUCHESNE gave valuable assistance with the various experiments.

BUREAU OF PLANT INDUSTRY, SOILS AND AGRICULTURAL ENGINEERING
BELTSVILLE, MARYLAND

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BREEDING BEHAVIOR AND CHROMOSOME NUMBERS IN PROGENIES FROM TWIN AND TRIPLET PLANTS OF POA PRATENSIS[1]

ETLAR L. NIELSEN

Introduction

Many workers have observed that *Poa pratensis* L. develops a high proportion of polyembryonic seeds, and several reports have been published concerning the appearance of mature plants developed therefrom. There are few data, however, relative to the breeding behavior of the plants. It is the purpose of this paper to set forth additional observations on this problem as well as to correlate them with chromosome numbers.

Review of literature

VUKULOV (**10**) found that 5.6% of the total number of seeds of *P. pratensis* studied were polyembryonic. Among other congeners studied were *P. palustris* L. with 0.8%, *P. nemoralis* L. with 0.7%, *P. compressa* L. with 0.3%, and *P. trivialis* L. with 0.13% polyembryonic seeds. Excellent illustrations of twin and triplet seedlings accompanied the data.

The sizes of stomatal openings within each of five sets of twins of *P. pratensis* having unequal chromosome numbers were compared with those of two sets of twins with equal chromosome numbers (**8**). There appeared to be a positive correlation between chromosome numbers and the lengths of the stomata, but the number of comparisons possible was not sufficiently large to permit NISSEN to arrive at reliable conclusions.

ÅKERBERG (**1**) obtained an average of 2.9% polyembryonic seeds in *P. pratensis*. Apomictic types considered in other studies averaged 11.2%, sexual ones 3.4%, and 37 collections of diverse origin 9.8% polyembryonic seeds. Approximately 13% of thirty-two twin sets consisted of divergent members, and in another trial approximately 7% of two hundred and sixty-two plants (apparently one hundred and thirty-one sets) were dissimilar.

About one half of thirty-one sets of twin seedlings of *P. pratensis* examined

[1] Results of co-operative studies between the Division of Forage Crops and Diseases, Bureau of Plant Industry, Soils and Agricultural Engineering, and the Wisconsin Agricultural Experiment Station, Madison. The statistical computations were made by personnel of the Statistical Laboratory of the College of Agriculture.

by SKOVSTED (9) had the diploid chromosome number, the remainder having deviating numbers. The seedlings of seven of the sets with equal numbers were considered to be of equal size, two of almost equal size, and seven others had pronounced differences. The seedlings of the fifteen other sets that had members with deviating chromosome numbers were classified as follows: three pairs were equal in size, three other pairs were almost equal in size, and nine pairs differed markedly within sets. Some of the latter sets were diploid-triploid. There did not appear to be any consistency as to which member was the more vigorous. One set was considered to be haploid-diploid, the haploid being the less vigorous as a seedling.

MÜNTZING (6) examined two hundred and seventy sets of *P. pratensis* twins. Eighteen plants were triploids, two were haploids, and five other deviating numbers were observed. He found that approximately 9% of all the plants had deviating chromosome numbers. He had concluded earlier (5) that usually the members of a twin pair have the same chromosome number but that sometimes one of the twins may be triploid to the diploid. The triploid plants were definitely more vigorous than their diploid mates. He suggested the hypothesis that in some of the Gramineae there may occur two or sometimes three macrospore mother cells. As a rule both of the embryo sacs derived from these macrospore mother cells are reduced, but sometimes one member may be diploid and the other haploid. Fertilization of the egg of each of these would consequently give rise to the diploid-triploid relationship. Likewise when haploid embryo sacs are present, the fertilization of the egg of one may stimulate the other to develop parthenogenetically so that haploid-diploid pairs would appear. Following further investigations (7) he reported that a triploid ($3n$) twin was usually less productive than its mate with the lower diploid ($2n$) chromosome number. It appeared that the triploid plant needed a longer time to reach maturity. Also, members with higher numbers had thicker and broader leaves than their corresponding twins with lower numbers. No difference could be found between plants in certain chemical characteristics. Pollen fertility was usually greater in the higher member of the set, and it seemed as though a change from the normal chromosome number to the triploid condition involved a change from a predominantly apomictic type of seed formation to a more or less pronounced degree of sexuality.

Several sets of twins and triplets were isolated by KIELLANDER (4) from an open-pollinated *P. pratensis* progeny ($2n = 72$) that had been completely uniform through three generations. Among these were two aberrant forms for the progeny, a twin with $2n = 40$, a triplet with $2n = 18$ that resembled *P. trivialis* ($2n = 14$). The $2n = 18$ triplet was partially fertile and produced offspring with the diploid number of chromosomes ranging from 15 to 19, the average number being 16.

Approximately 7% of the seed samples taken by BRITTINGHAM (3) from plants of 115 progenies were polyembryonic. The range in percentages varied from 0 to 31.6. The highest frequency of seeds with triplet embryos from a single plant was 4.3%. Sixty-nine twin sets and four triplet sets were grown to maturity. Approximately 17% of the sets were considered to be dissimilar, and it was concluded that the variability was significantly higher among plants derived from polyembryonic seeds than that among

plants derived from single-embryo seeds. Although this difference was noted, the nature of variation was considered essentially the same. Likewise, no association was found between plant type or manner of origin and the frequency of polyembryony in the species.

Material and methods

Two groups of progenies have been analyzed for the frequencies of aberrants, i.e., deviations from the predominant type. Twenty-four paired progenies (group I) from twins and two from triplets were established in the field in 1942 and subsequently studied in 1943. The parents of this group had been isolated by F. W. TINNEY.

Sixty-two sets of twins and four sets of triplets (group II) were isolated in March, 1942, from diverse lots of seed germinated in connection with other studies. The seedlings isolated cannot be taken as a random sample of Kentucky bluegrass twins, since an effort was made to isolate more dissimilar pairs with the consequence that numerous apparently identical seedling sets were not propagated. The plants of selected sets were separated and later space-planted into the field in May. These were classified as to their morphological similarity or dissimilarity during their first fruiting year. Individual panicles were harvested separately from each plant. Fifteen sets of twins and three sets of triplets were selected for further study. The basis for selection of these was morphological similarity or dissimilarity among the members of a given set. Derived progenies of twenty-four plants each were established in the field in 1944, and these were subsequently analyzed for their frequencies of aberrants during the summer of 1945.

Results

GROUP I

The data for the frequencies of aberrants among progenies are given in table 1. The chi-square test for independence shows that in three sets the percentage of aberrants in the progeny of one member of the pair is significantly different from that in the other. YATES' (11) correction for continuity was used, since there was only one degree of freedom in each comparison. The frequency of aberrant plants in line 251 differed at the 1% level and in lines 275 and 279 at the 5% point. No difference was ascertainable among the triplet progenies.

GROUP II

THE PARENT PLANTS.—The original plant isolations from which progenies were developed were analyzed for vigor, aggressiveness, plant height, texture, and other similar characters in June, 1943. Forty-six per cent of the twins were considered identical. Fifteen of the thirty-one sets of twins isolated from seed lots producing more than one set of twin seedlings were classified as dissimilar and sixteen as similar. At least one individual of the respective set was dissimilar in two of the four sets of triplets.

SEEDLINGS FROM TWINS AND TRIPLETS.—The germinating seed lots from the different pairs of group II parents were compared, relative to the number and vigor of the seedlings produced, during the seedling stage. Results are summarized in table 2.

The seed of one plant of each of two twin sets was completely sterile, no seedlings developing from either plant. That from their mates produced many and intermediate numbers of seedlings, respectively.

TABLE 1

NUMBERS OF PLANTS AND PERCENTAGES OF ABERRANTS IN PROGENIES FROM TWINS AND TRIPLETS OF POA PRATENSIS

Progeny no.	*a*-Progeny*		*b*-Progeny*		*c*-Progeny*	
	No. of plants	Aberrant (%)	No. of plants	Aberrant (%)	No. of plants	Aberrant (%)
237	24	4.2	24	0.0		
239	24	0.0	24	0.0		
241	24	4.2	24	4.2		
243	24	4.2	24	0.0		
245	24	4.2	24	4.2		
247	24	0.0	24	8.3		
249	24	8.3	24	4.2		
251	24	4.2	24	50.0		
253	24	4.2	24	4.2		
255	24	4.2	24	12.5		
257	24	4.2	24	0.0		
261	24	4.2	24	4.2		
263	24	0.0	24	0.0		
265	24	0.0	24	0.0		
267	24	8.3	24	4.2		
269	24	0.0	24	4.2		
273	24	8.3	24	4.2		
275	24	0.0	24	20.0		
277	24	4.2	24	8.3		
279	24	0.0	24	16.7		
281	24	8.3	24	16.7		
285	24	33.3	24	33.3		
287	24	4.2	24	8.3		
289	8	0.0	5	0.0		
291	15	0.0	24	0.0	24	0.0
294	24	4.2	24	0.0	24	8.3

* *a*, *b*, and *c* indicate progenies from different members of twin and triplet sets.

TABLE 2

RELATIVE NUMBER AND VIGOR OF SEEDLINGS FROM SINGLE PANICLES OF TWIN PLANTS OF POA PRATENSIS

No. of seedlings per set				Vigor of seedlings per set			
b-Plant	*a*-plant			*b*-Plant	*a*-plant		
	Many	Intermediate	Few		Good	Fair	Poor
Many	4(4)/8*	7(6)/13	5(2)/7	Good	5(6)/11	11(7)/18	0(3)/3
Intermediate		4(9)/13	5(3)/8	Fair		6(9)/15	6(3)/9
Few			5(6)/11	Poor			2(2)/4

* The first figure is for comparisons of similar plants of sets of twins; the italicized figure (in parentheses) is of dissimilar members of sets. The totals of each comparison in each class are given following the slanting line.

Significant differences were not found between the number of seedlings developed from the different sets. Approximately half of the total seed lots germinated similarly, and the vigor of the seedlings was essentially the same.

plant type or vigor. There is no significant difference between the germinability of seed of either type of average or better vigor. However, there is a tendency for nonvigorous plants to produce inferior numbers of viable seeds

TABLE 3

VIGOR AND SIMILARITY OF MEMBERS OF TWIN AND TRIPLET SETS OF POA PRATENSIS, SIMILARITY OF PLANTS OF THEIR PROGENIES, GERMINATION RECORDS, AND NUMBER OF PLANTS AND PERCENTAGE OF ABERRANTS IN PROGENIES

No.	Vigor of plants			Similarity of		Seedling*						*a*-Progeny†		*b*-Progeny†		*c*-Progeny†	
						Av. no.			Vigor								
	a	*b*	*c*	Sets‡	Progenies‡	*a*	*b*	*c*	*a*	*b*	*c*	No. of plants	Aberrant (%)	No. of plants	Aberrant (%)	No. of plants	Aberrant (%)
36-1	P	P		−	−	F	F		P	P		17	41.2	18	72.2		
252-1	G	G		+	+	M	F		F	P		20	10.0	23	13.0		
252-2	G	G		−	−	I	I		F	F		23	30.4	23	43.5		
252-4	F	F		+	+	F	VF		F	P		22	31.8	21	23.8		
292-1	G	G		+	+	I	I		G	F		22	4.5	21	4.8		
292-2	G	G		+	+	F	F		F	F		20	10.0	20	25.0		
358-1	F	P		−		M	o		P	−		23	52.2	Sterile			
397-1	G	G		+	+	F	I		F	F		21	23.8	20	20.0		
397-3	G	P		−		I	o		F	−		20	25.0	Sterile			
540-1	G	F		−	−	M	I		G	P		22	27.3	20	100.0		
353-1	G	F		−	−	F	F		P	P		23	4.3	22	4.5		
587-3	P	P		+	+	F	F		P	P		21	14.3	27	13.6		
587-4	F	G		−	−	M	M		F	G		18	33.3	22	40.9		
652-1	F	P		−	−	I	I		F	P		22	100.0	21	66.6		
652-2	G	F		−	−	I	I		F	F		22	36.4	21	23.8		
26-1	F	F	P	−	−	F	M	F	P	F	P	18	18.7	23	17.4	23	4.3
34-1	F	F	F	+	+	I	I	F	F	F	F	14	21.4	20	30.0	16	37.5
599-1	G	G	G	−	−	F	I	F	F	F	F	21	9.5	22	9.1	20	40.0

* Average number and vigor of seedlings: M, many; I, intermediate; F, few; G, good; F, fair; and P, poor vigor.
† *a*, *b*, and *c* indicate progenies from different members of twin and triplet sets.
‡ Plus (+) symbols indicate similarity; minus (−) symbols dissimilarity.

The data for germination of seed lots from three sets of triplets are included in table 3. Many seedlings resulted from the seed lots from all members of the fourth set. Those from two plants were of good vigor, whereas those from the third were only fair in this respect.

A comparison (table 3) of germination records of plants of similar sets of twins with those of dissimilar sets shows that the number and the vigor of seedlings are characteristics generally unrelated to that develop into seedlings of low vigor. The data for sets of triplets are not sufficiently extensive to permit generalization.

PROGENIES.—Data for the second set of progenies from twin and triplet plants of Kentucky bluegrass are given in table 3. Results were analyzed similarly to those for the 1942 progenies. The chi-square test for independence indicated that the frequency of aberrants in progenies from three sets differed significantly.

These include sets 540-1, 599-1, and 652-1. Inspection of these data also indicates that the predominant plant types of any given set of progenies may be similar or different, a fact that a statistical test of this type cannot be used to determine. Likewise, the two progenies grown from the mates of sterile plants showed that one of these individuals was predominantly apomictic and the other intermediate or tending slightly toward the sexual mode of reproduction.

CHROMOSOME NUMBER.—Plants of six of the progenies from sets of twins and two of the progenies from triplets were examined for their chromosome numbers. The chromosome number reported for the predominant type is, in all cases, based upon approximations in one or two members of a progeny and a detailed analysis and check of one plant. Two or more plants of each progeny were examined. The chromosome numbers of two additional sets of twins were examined, these being the sets of which the seeds of one individual were sterile. Aceto-carmine smear preparations were used, and, with one exception (that at diplotene), diakinesis figures were studied for the determinations. It may be seen from figures given here, and from those of previously published papers, that the chromosomes of *P. pratensis* are small, often numerous, and have strong tendencies to bunch. The chromosome counts are summarized in table 4. Those of the higher numbers, particularly, should be considered to be only approximations of the actual number of a given biotype or line.

SEXUAL COMBINATIONS

Two sets of progenies from twins of this category were examined. Members of the first pair (358-1, table 3) to be considered were very different in their morphological characteristics. One plant (*a*) was of fair vigor, 15 inches high, with leaves of medium length, and generally open panicles (fig. 27). The second plant (*b*) was very weak but survived the first winter after it was transplanted into the field. It continued to be weak; at flowering time the following summer, it reached a maximum height of 6 inches, had short leaves, and developed three short, compact panicles. One panicle was left to mature seed and the other two were fixed for cytological examination. This plant failed to survive the second winter. The *a*-plant (meiotic complement $\pm 27_{II} + 2_{I}$, fig. 1) was fertile, and the progeny of this plant contained approximately 52% aberrants. The *b*-plant was completely sterile. Its anthers were partially defective, but sufficient microspore mother cells developed so that at least some data were obtained. $8_{II} + 12_{I}$ (fig. 2) were usually present at diakinesis. Occasionally others had 6, 7, and 9 bivalents, with corresponding deviations of univalents. Fourteen chromosomes pass to

TABLE 4

APPROXIMATE CHROMOSOME NUMBERS IN PLANTS ISOLATED, OR IN PLANTS OF PROGENIES FROM TWINS AND TRIPLETS OF POA PRATENSIS*

Twin or triplet set number	*a*-progeny	*b*-progeny	*c*-progeny
358-1	56†	28†	
652-1	56	56	
587-3	56	57	
252-4	57 (80)‡	59 (98)‡	
587-4	86	58	
397-3	58†	117†	
540-1	84	56	
599-1	84	84	114 (86)‡
34-1	110	108	109

* Numbers calculated from meiotic complements observed approximately at diakinesis.

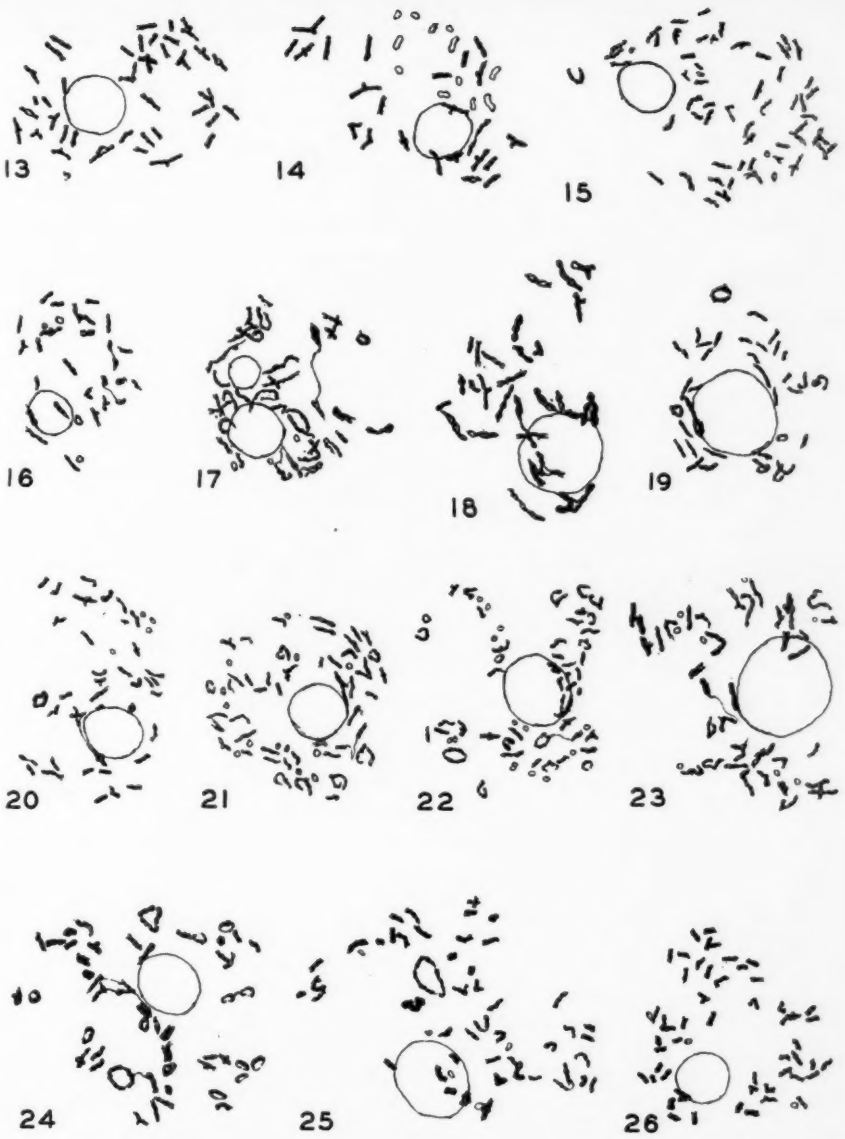
† Counts made upon twin plants isolated, of which one member of set proved to be sterile.

‡ Numbers in parentheses are of aberrants in progenies.

each pole at anaphase (figs. 3, 4). A chromosome fragment is sometimes present at this stage. Comparative data for other stages are given in table 5. Chromosome bridges and, in at least one case, a dicentric bridge with an acentric fragment were present. Additional observations concerning macrosporogenesis in the plant are given in the companion paper.

The data suggest origins of one or two methods of formation of twins: (1) the stimulation of a synergid of a reduced embryo sac by the act of fertilization of the contiguous egg or (2) the stimulation of the egg of one embryo sac of an ovule

FIGS. 1–12.—Meiotic complements in plants of *Poa pratensis:* Fig. 1, 358-1*a*. $27_{II}+2_{I}$. Fig. 2, 358-1*b*. $8_{II}+10_{I}$. Fig. 3, 358-1*b*. Anaphase$_{I}$. Fig. 4, 358-1*b*. Anaphase$_{II}$. Fig. 5, 652-1*a*- plant 3. 28_{II}. Fig. 6, 652-1*b*- plant 3. $2_{III}+16_{II}+17_{I}$ (?). Fig. 7, 587-3*a*- plant 3. 28_{II}. Fig. 8, 587-3*b*- plant 3. $28_{II}+1_{I}$. Fig. 9, 252-4*a*- plant 3. $27_{II}+3_{I}$. Fig. 10, 252-4*a*- plant 8. $1_{IV}+3_{III}+23_{II}+10_{I}$. Fig. 11, 252-4*b*- plant 5. $1_{III}+23_{II}+7_{I}$. Fig. 12, 252-4*b*- plant 2. $55_{II}+2_{I}$.



FIGS. 13–26.—Meiotic complements in plants of *Poa pratensis:* Fig. 13, 587-4*a*- plant 3. 43_{II}. Fig. 14, 587-4*b*- plant 4. $23_{II}+12_{I}$. Fig. 15, 397-3*b*. $57_{II}+3_{I}$. Fig. 16, 397-3*a*. $1_{III}+24_{II}+4_{I}$. Fig. 17, 540-1*a*- plant 3. $2_{III}+36_{II}$. Fig. 18, 540-1*b*- plant 6. 28_{II}. Fig. 19, 599-1*a*- plant 4. 42_{II}. Fig. 20, 599-1*b*- plant 6. 42_{II}. Fig. 21, 599-1*c*- plant 5. $1_{III}+48_{II}+13_{I}$. Fig. 22, 599-1*c*- plant 6. $1_{IV}+1_{III}+42_{II}+16_{I}$. Fig. 23, 599-1*c*- plant 14. $39_{II}+8_{I}$. Fig. 24, 34-1*a*- plant 5. $1_{VI}+1_{IV}+1_{III}+42_{II}$. Fig. 25, 34-1*b*- plant 9. $1_{III}+49_{II}+4_{I}$. Fig. 26, 34-1*c*- plant 7. $1_{III}+50_{II}+3_{I}$.

as a result of fertilization of the egg of the second embryo sac.

The second set of twins (652-1) was also made up of quite dissimilar members (fig. 28). One plant (*a*) was vigorous, somewhat aggressive, medium in texture, and at maturity reached a height of 29 inches. The second plant was of poor vigor, nonaggressive, medium texture, and, at maturity, was 19 inches tall. All members of the progeny developed from the *a*-plant differed from one another in morphology. Representatives of this progeny contained approximately 28_{II} at diakinesis (fig. 5). Of the progeny from the *b*-plant, 67% were classified as morphologically dissimilar. Generally they were inferior to those of the sister *a*-progeny in vigor, leafiness, and height. Members of this progeny averaged about $2_{III} + 16_{II} + 17_{I}$ at diakinesis, with multiple associations of three chromosomes being common (fig. 6).

It would appear that this set of twins arose through the fertilization of an egg and a synergid of one embryo sac or, possibly, through the fertilization of the eggs of two different embryo sacs in the same nucellus. Noncompatibility as evidenced by frequent unpaired chromosomes of the *b*-progeny plants may have been sufficiently effective to reduce the general vigor of the plants of the progeny.

FIGS. 27–28.—Mature twin plants of *Poa pratensis:* Fig. 27, *left*, 358-1*b*; *right*, 358-1*a*. Fig. 28, *left*, 652-1*a*; *right*, 652-1*b*.

TABLE 5

AVERAGE NUMBER OF LAGGARDS PER SPOROCYTE AT METAPHASE I, ANAPHASE I, TELOPHASE I, DIAD, AND MICRONUCLEI PER QUARTET IN HAPLOID-DIPLOID POA PRATENSIS TWIN PLANTS

Plant	*n*	Metaphase I	Anaphase I	Telophase I	Diad	Quartet
358*a*..	28	1.88	2.28	1.00	0.84	1.32
358*b*..	14(+*f*)	1.00	1.00	0.80	0.20	0.44

PREDOMINANTLY APOMICTIC COMBINATIONS

SIMILAR SETS OF TWINS.—Two sets of twin progenies wherein the predominant plant type was similar were examined for chromosome numbers. Members of set 587-3 were essentially equal in frequencies of aberrants, these being 14.3 and 13.6%, respectively. Generally plants from the *a*-parent had 28 bivalents whereas those from the *b*-parent had $28_{II} + I_{I}$ (figs. 7, 8, 29, 30) at meiosis.

Plants of the predominant type from the second set of twins (252-4), with approximately 32 and 24% aberrants, had

$\pm 27_{II} + 3_I$ (fig. 9) and $\pm 1_{III} + 23_{II} + 7_I$ (fig. 11),[2] respectively. One aberrant from the *a*-plant had $\pm 1_{IV} + 3_{III} + 23_{II} + 10_I$ (fig. 10), or an equivalent of 35 bivalents at meiosis. An aberrant plant of the mate or *b*-progeny had about $55_{II} + 2_I$, or the equivalent of 56 bivalents (fig. 12).

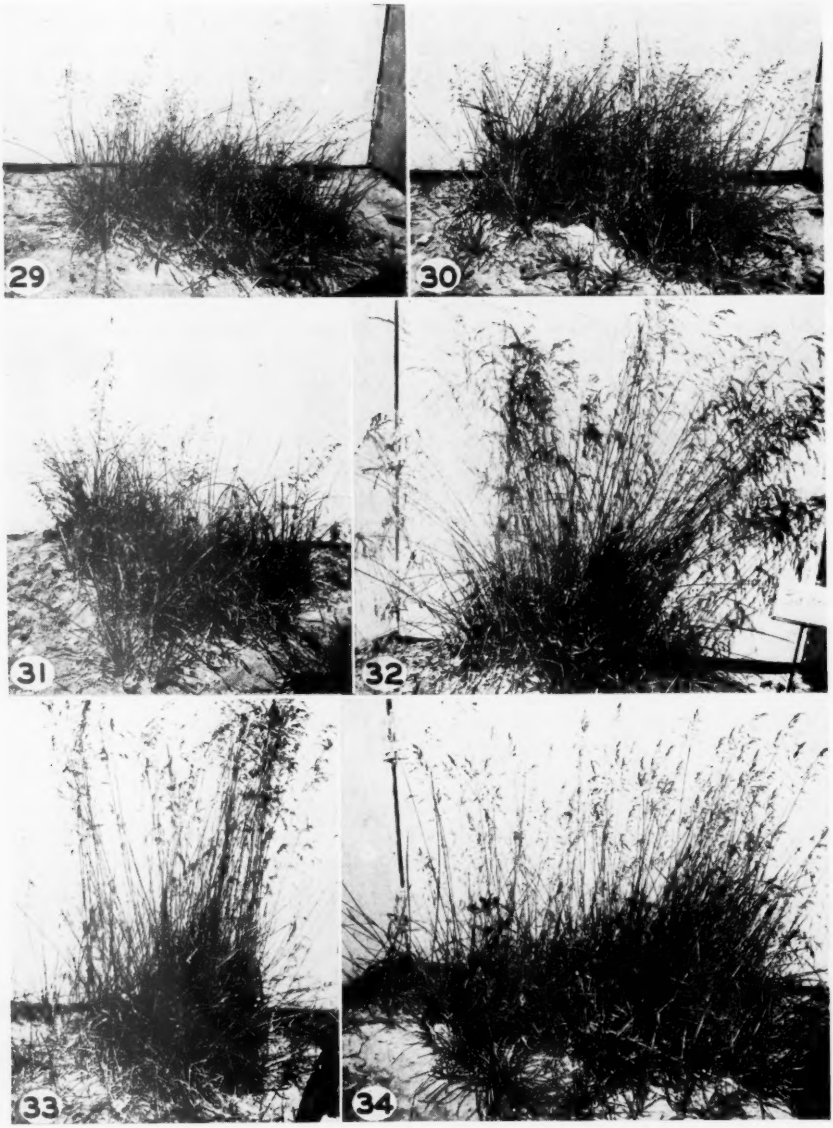
These observations suggest that twin embryos developed in ovules of the parents of 587-3 and 252-4 without fertilization of the eggs. Both twin sets developed progenies of low and essentially equal frequencies of aberrants. In addition, however, seedlings derived following fertilization were represented in both of these progenies. The aberrant in the *a*-progeny (252-4*a*- plant 8) apparently arose through the fertilization of the egg cell of a somatic aposporous embryo sac with a male gamete carrying approximately the reduced number of chromosomes ($n = 28$). The aberrant of the *b*-progeny examined (252-4*b*- plant 2), with $\pm 55_{II} + 2_I$, is somewhat more difficult to explain. The observations suggest either fertilization by an unreduced gamete or a somatic doubling of the dividing zygote nucleus.

Based upon progeny behavior, four other sets of twins were probably of this type (252-1, 292-1, 292-2, and 397-1).

DISSIMILAR SETS OF TWINS.—Members of two sets of progenies, wherein the predominant type in a given progeny was unlike that of their mates, were examined. The members of the sets of twins 587-4 were markedly dissimilar, differing in growth habit and aggressiveness, texture or coarseness of both culms and leaves, general vigor, and plant height (figs. 31, 32). Although progenies developed from twin plants did not differ significantly in their frequencies of aberrants (40.9 and 33.3%), the morphological characteristics of the predominant type of two progenies differed greatly. Members of the *b*-progeny had about $23_{II} + 12_I$ (fig. 14), whereas those of the other had approximately 43_{II} (fig. 13). This is interpreted to mean that the ovule of the plant wherein these twins developed contained either two aposporous embryo sacs, of which the egg of each formed a functional embryo, or that it contained an embryo sac that also arose through somatic apospory. One of the synergids could have developed because of the stimulus given it by the fertilization of the egg. In either case it appears that a second egg or a synergid developed parthenogenetically because of the stimulus set up by the fertilization of the other functional member in the ovule. Although the end results would be the same, the last-mentioned alternative seems the more plausible, based upon the relative frequencies of multiple embryo sacs observed.

The observations on the second set (397-3) are somewhat more difficult to interpret. In this set the two members of the twin set differed markedly in vigor, aggressiveness, texture, and plant height, the member designated as *b* being, in all characters mentioned, inferior to those of the mate (*a*). The relative heights were 10 and 23 inches, and the chromosome numbers about $57_{II} + 3_I$ (fig. 15) and $\pm 1_{III} + 24_{II} + 4_I$ (fig. 16), respectively. Plant *b* proved to be sterile as determined by seed germination for subsequent progeny tests; plant *a* produced numerous functional seeds and the mature progeny therefrom had 25% aberrant plants. MÜNTZING (5) suggested that the occurrence of tetraploids among twins most probably implies somatic doubling after fertilization or possibly

[2] It was not possible in the material examined to determine whether the multiple associations of chromosomes were due to homologies or to catenations. They are therefore considered as multiple associations of either or both types.



FIGS. 29–34.—Members of progenies from twins in *Poa pratensis:* Fig. 29, 587-3*a*- plant 10. Fig. 30, 587-3*b*- plant 14. Fig. 31, 587-4*a*- plant 20. Fig. 32, 587-4*b*- plant 14. Fig. 33, 652-2*a*- plant 2. Fig. 34, 652-2*b*- plant 4.

doubling at the beginning of embryo development if this has started parthenogenetically. Other possible explanations for this condition should be mentioned. Only one of these appears at all likely to occur. The ovule from which this polyembryonic seed developed perhaps contained two embryo sacs that had arisen by somatic apospory. The maternal tissue in this case probably contained about 56 chromosomes. One egg of these embryo sacs was stimulated to develop parthenogenetically and eventually resulted in the fertile plant. The second egg may have been fertilized either by an unreduced gamete or by a high polyploid gamete carrying approximately the same chromosome number as the egg. Although pairing was evident, subsequent interference may have resulted in complete sterility. It is quite unlikely that the combination represents a true haploid-diploid relationship of embryos developed from a fertilized egg and a synergid of a single embryo sac. Judging from other experiences the haploid would either be sterile or, if fertile, the progeny therefrom would show considerable segregation. The opposite is true in this case. It is likely that the first of the alternatives, that suggested by MÜNTZING, is the most plausible explanation of the case in question. However, the second alternative is possible but is not likely to be of ɔmon occurrence. Restitution nuclei were not observed, or so recognized, in any abundance, and very high polyploids were comparatively uncommon in the material examined.

The progeny behavior of three other sets of twins (252-2, 353-1, and 652-2 [figs. 33, 34]) suggests similarity to 587-4. Members of two of these sets, however, tend to produce fairly high proportions of aberrant offspring.

MIXED SEXUAL AND APOMICTIC COMBINATIONS

The progeny of one member of a set of twins (540-1) was predominantly uniform (27.3% aberrants), whereas the progeny from its mate was extremely diverse, no two plants being entirely alike. In this case plants of the predominant type of the former contained approximately $2_{III} + 36_{II}$ (fig. 17), whereas plants of the sister progeny had 28_{II} (fig. 18). Comparatively few plants of the species are either entirely apomictic or entirely sexual in their modes of reproduction, the majority of plants being about 20% sexual. Plants of intermediate tendencies are relatively common. It has been observed that there sometimes occurs in the same ovarian primordium a functional macrospore mother cell and a densely cytoplasmic nucellar cell similar to those found in apomictically reproducing forms. Usually one of these, or its descendants, will fail to survive, particularly so when one or the other is of advanced development. When the development is synchronous or essentially so, embryos may form in both embryo sacs. The difference between the pair of twins in question (540-1), and their progenies, seems to be explained best in this manner. If the egg of the embryo sac having the gametophytic chromosome number is fertilized, a progeny of diploid plants would result. Because of the apparent heterozygosity of plants of *P. pratensis*, a highly or even totally diverse progeny would be expected. This is based upon the assumption that all fertile florets of a given plant contained reduced gametophytes, the eggs of which were fertilized. The egg cell of the somatic aposporous embryo sac might be stimulated to develop either a diploid embryo parthenogenetically or, if fertilization by a re-

duced gamete occurs, an embryo of triploid constitution.

The breeding behavior of one set of twins (36-1) does not appear to fall definitely into any one single class. One plant of this set produced 41% aberrants compared with 72% for its mate. It appears evident that both these plants carry intermediate to strong tendencies toward sexuality, and it is equally evident that, though these tendencies are strong, neither plant is entirely sexual or strongly apomictic. In default of any better classification of the set, it is included here.

Triplets

Similar sets.—The predominant plant type was similar in all three progenies from one set of triplets (34-1). The progenies contained 21.4, 30.0, and 37.5% aberrants, respectively, for *a*, *b*, and *c*. Representative plants of the predominant type of each of these progenies contained about the following chromosome complements: $1_{VI} + 1_{IV} + 1_{III} + 42_{II}$ (fig. 24), $1_{III} + 49_{II} + 4_{I}$ (fig. 25), and $1_{III} + 50_{II} + 3_{I}$ (fig. 26), respectively. These are interpreted to indicate the development of the triplet set from the eggs of three aposporous embryo sacs in the same ovule; or the development of embryos from all members of the egg apparatus of a single sac; or an intermediate combination of two embryos arising from members of one embryo sac and one from those of a second embryo sac. In any event, it appears unlikely that fertilization of the egg of any one of these took place. Had such fertilization been effected, there would perhaps have been a phenotypic as well as a cytologic difference expressed in the progeny from one member. There is evidence that fertilization is not necessary for embryo development in apomictic biotypes of *P. pratensis* (2).

Dissimilar sets.—Progenies from two members of a set (599-1) of triplets were predominantly uniform and morphologically similar. A few of the plants (9–10%) of each of these progenies were aberrant. Sixty per cent of the plants from the third triplet were generally more vigorous and differed morphologically from those of the sister members of the set. Characteristically, plants of the two similar members of the set had chromosome constitutions approximating 42_{II} (figs. 19, 20) compared with equivalents of 57_{II} to 59_{II} (figs. 21, 22) in individuals of the third progeny. This combination is essentially similar to several cases previously indicated for twins. The mode of origin of this set of triplets is a matter of conjecture. It is most probable that they arose from at least two embryo sacs, the only basis for this surmise being, however, that in no instance in this or in earlier reported studies have all members of the egg apparatus of a single embryo sac been seen to develop. Plant 14, one of the aberrants of the progeny (599-1) with 57–59 bivalents, had approximately 42_{II} (fig. 23). The most logical explanation of this plant would appear to be the fertilization of a reduced egg (56_{I}) with a male gamete carrying 28 chromosomes. This would result in a plant with $2n = \pm 84$, which corresponds with the count made.

Discussion

Data presented in this paper clarify, in part at least, some of the problems that have arisen in connection with the chromosome numbers and the highly variable gross morphology of the different biotypes of *P. pratensis*. The most recent tabulation (2) of somatic chromosome

numbers reported for the species indicates a range of from 24 to ±124, exclusive of calculated complements. In the same paper ÅKERBERG reported that a biotype from Germany had $2n = \pm 130$. Twenty-five of the numbers were euploid, the remaining 127 being aneuploid. Graphically the distribution of the more definite chromosomal counts is a skewed trimodal curve with apices in the 49–56, 63–70, and 84–91 chromosome-number classes. These apices each represent 38, 29, and 17 determinations, respectively.

The combination of both sexual and apomictic modes of reproduction, coupled with free interpollination of biotypes and apparently weak genetic barriers, with all their possible interactions, appears to be a sufficient explanation for the chromosome and morphological diversity of *P. pratensis*.

Haploid plants of this species have not been extensively studied. Most reports concern chromosome number only. The single plant of this type ($n = 14$) considered here was very weak. Subsequently the plant proved to be sterile. This is not always the case. MÜNTZING (**7**) found a haploid ($n = 36$) that produced a few seeds after self-pollination. Twenty-one of twenty-nine resulting plants examined had 36 chromosomes, one each had 29, 34, 37, 49, and 52, and three had 35. Plants from seed of florets allowed access to open-pollination generally had from 32 to 58 chromosomes. An analogous case was also presented for *P. alpina*. A somewhat similar instance has been reported by KIELLANDER (**4**) for open-pollinated *P. pratensis*. These examples are sufficiently illustrative of the diversity that may arise in progenies from plants with low chromosome numbers.

Aside from the data given in this paper, the writer is aware of chromosome counts only for the exceptionally high polyploids of the species. The reduced vigor and the accompanying sterility of the plant reported here suggest that this approaches or has reached the chromosomal limits wherein fertility might be expected to exist. Examination of the germination records of other plants (*252-4b* and *599-1c*) with high chromosome numbers tends to support this opinion. In both cases relatively few seedlings developed from the seed of individual panicles. The seedlings of *599-1c* were classified as of fair vigor and those of *252-4b* as poor.

The synthesis of new chromosome and genetic combinations between the above-indicated extremes can apparently be accomplished more or less successfully without serious detriment to the fertility of the resulting new combination. Apomixis, accompanied by sexual reproduction in various proportions of florets, thus affords in some measure a mechanism for maintaining an unstable genetic and chromosomal condition in the biotypes. The end result of such a condition of reduction and accumulation of genetic factors and chromosomes can only be a multiplicity of forms and considerable diversity in breeding behavior.

Summary

Two groups of twin and triplet sets are considered. Twenty-four pairs of twins and two sets of triplets (group I) were grown to maturity from seedlings. The frequency of aberrants in progenies from three sets of twins was found to differ significantly. No such difference occurred between the progenies from the other twenty-one sets of twins or between those from the two sets of triplets.

Forty-six per cent of a nonrandom

population (group II) of twin and triplet sets differed in morphological characteristics. No significant differences were found between the number and the vigor of seedlings from members of multiple sets. Likewise, no difference occurred between the same characters for identical compared with dissimilar sets.

The frequency of aberrant plants was significantly different in progenies from two of the twin and one of the triplet sets. The gross morphology of the predominant plant type in progenies from seven twin and two triplet sets was greatly different.

It was concluded from the breeding behavior and the chromosome number in plants of progenies, and from two sets of twins, that multiple embryos had arisen in the following manners: (1) from the egg and a synergid of an embryo sac carrying the reduced chromosome number, one member of the egg apparatus developing parthenogenetically because of the stimulative effect of fertilization of the other; (2) from two embryo sacs in which the eggs (or an egg and a synergid) carrying the gametophytic chromosome number had been fertilized; (3) from the unfertilized eggs, or from an egg and a synergid, of embryo sacs that arose by somatic apospory; (4) from eggs (or the egg and a synergid of one sac) of embryo sacs developed through somatic apospory, one forming a diploid embryo and the other a tetraploid by somatic doubling of the dividing zygote; (5) from two egg cells or from an egg and synergid that arose by somatic apospory, one of which was fertilized, the other developing parthenogenetically; and (6) from the fertilized egg derived from a reduced macrospore and from an egg of an aposporous embryo sac.

The observations are discussed in relation to known breeding behavior of biotypes of the species and to the morphological and chromosomal variation in naturally occurring populations of the species.

The writer gratefully acknowledges the suggestions made by colleagues during the progress of this study.

DEPARTMENT OF AGRONOMY
UNIVERSITY OF WISCONSIN
MADISON, WISCONSIN

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THE ORIGIN OF MULTIPLE MACROGAMETOPHYTES IN POA PRATENSIS[1]

ETLAR L. NIELSEN

Introduction

There has been some divergence of opinion concerning the origin of multiple embryos in *Poa pratensis* L. This paper will consider briefly the scope of the problem and indicate the conclusions arrived at by other workers. Additional data have been obtained and are presented so that integrated conclusions may be reached. Confirmatory evidence from breeding behavior and chromosome numbers in plants from twins and triplets has been obtained and is presented for most of the types of gametophytes (and subsequent embryo development) observed or postulated from these cytological studies (**10**).

Review of literature

Individual seeds of *P. pratensis* sometimes contain from one to three embryos (**14**). ZIMMERMAN accredits a Dr. HEINRICH with previously observing a large number of twin embryos and, in one case, three distinct seedlings developing from a single seed. ZINN (**15**) also noted polyembryonic seeds in *P. pratensis*, *P. nemoralis* L., and *P. compressa* L.

Histologic sections of the developing ovules of *P. pratensis* were interpreted by NISHIMURA (**11**) to indicate the formation of plural embryos from a massive suspensor. "Two or even three buds may develop from the suspensor apparatus." He also "observed where the nucellar cells in the vicinity of the micropyle gave rise to two or three bud-like outgrowths." These were seen in the antipodal region wherein the massive suspensor was also a characteristic feature. Also, nucellar cells lateral to the embryo sac might form embryonic buds. Frequently the growth of the normal embryo sac was inhibited by the growth of the other embryos. These and other similar phenomena were thought to be associated with mechanical injury due to insect attack or to oviposition.

ANDERSEN (**2**) found that twin embryo sacs arose from sister macrospores in *P. pratensis*. This work was done before the occurrence of apomixis had been suggested as occurring in *Poa* species.

A haploid-diploid relation in *P. palustris* L. (*P. serotina* Ehr.) with 14 and 28 chromosomes, respectively, was reported by KIELLANDER (**6**), who concluded that in this case the haploid developed from a reduced egg cell. Later he (**7**) isolated a set of triplets of *P. pratensis*, one member of which had 18 chromosomes. This subhaploid was partially fertile and, after open pollination, gave plants with 15–19 chromosomes.

An evident difference between apomictic strains in their capacities to form several embryos was found by ÅKERBERG (**1**). Two seedlings from a single seed frequently developed at different times and sometimes were quite diverse in appearance. Later they might appear to be phenotypically alike. Two haploid aberrants ($n = \pm 24$ and ± 29) differed greatly from the normal *P. pratensis* plants.

[1] Results of co-operative studies between the Division of Forage Crops and Diseases, U.S. Department of Agriculture, Soils and Agricultural Engineering, and the Wisconsin Agricultural Experiment Station, Madison.

The frequency of seeds with twin and triplet embryos also varies with the North American strain of Kentucky bluegrass studied (3). Among the different ones observed, ARMSTRONG found that they produced 8, 11, 35, and 42%, respectively, of polyembryonic seeds. In his material he "frequently observed two EMC lying side by side in the prophase stage." These were thought to develop into sister macrospores.

TINNEY (13) found that two embryo sacs may arise independently from two differentiated nucellar cells, but he did not exclude the occurrence of haploids arising from reduced or haploid eggs or the development of triploids as a result of gametic union of an unreduced egg with a haploid male gamete.

ENGELBERT (4) stated that twin embryo "sacs so commonly found in polyploid, polymorphic *Poa* species are, as a rule, developed one from a macrospore, the other from an aposporous cell."

Any of the haploid macrospores of sexual plants of *P. alpina* L. is capable of developing into an embryo sac, with more than one developing occasionally (5). It was concluded that there was no reason to assume that somatic aposporous embryo sacs develop frequently in this species, as often is the case in *P. pratensis*. In the apomictic types of *P. alpina* the embryo sac is formed directly from the macrospore mother cell through mitosis, and, as a result, no reduced macrospores are formed.

Multiple embryo sacs in *P. pratensis* may result in haploid-diploid and in diploid-triploid twins, thus indicating, in one case, the completion of the reduction divisions to form macrospores and, in the other case, the fertilization of the egg of an apomictically formed sac (8).

The frequency of multiple embryo sacs varies from 0 to 63% in ovules of predominantly apomictic, intermediate, and sexual plants of Kentucky bluegrass (9). Apomictic types generally have higher percentages of multiple embryo sacs per floret than do sexual plants.

Material and methods

Fixations were made at 2-day intervals from the emergence of the tip of the panicle until anthesis, after which two additional fixations were made prior to seed ripening. The material selected for each fixation represented the apparently youngest to the most developed florets in several panicles. Later, spikelets of the various sizes and presumed ages were selected from each fixation for histologic study. The usual types of alcohol dehydration and clearing solutions were employed. Sections were cut at 12–25 μ, depending upon the size or age of the florets. Staining was effected with either Haidenhain's iron-alum or Delafield's haematoxylin.

Results

DEVELOPMENT FROM TWO MACROSPORE MOTHER CELLS.—Occasionally two adjacent macrospore mother cells were observed in the regular position in the nucellus (fig. 1). This was especially true of one plant. The reduction divisions occur and two lineal tetrads of macrospores form. Following the regular pattern of events for sexual Kentucky bluegrass plants, two embryo sacs eventually develop (fig. 2). The probable consequence of this type of origin of plural embryos would be two dissimilar plants. Progenies from these would be expected to be highly diverse in plant type.

DEVELOPMENT FROM TWO MEMBERS OF THE EGG APPARATUS.—This relationship perhaps is not widespread in *P. pratensis* owing in part to the frequency of apomictic reproduction in the species.

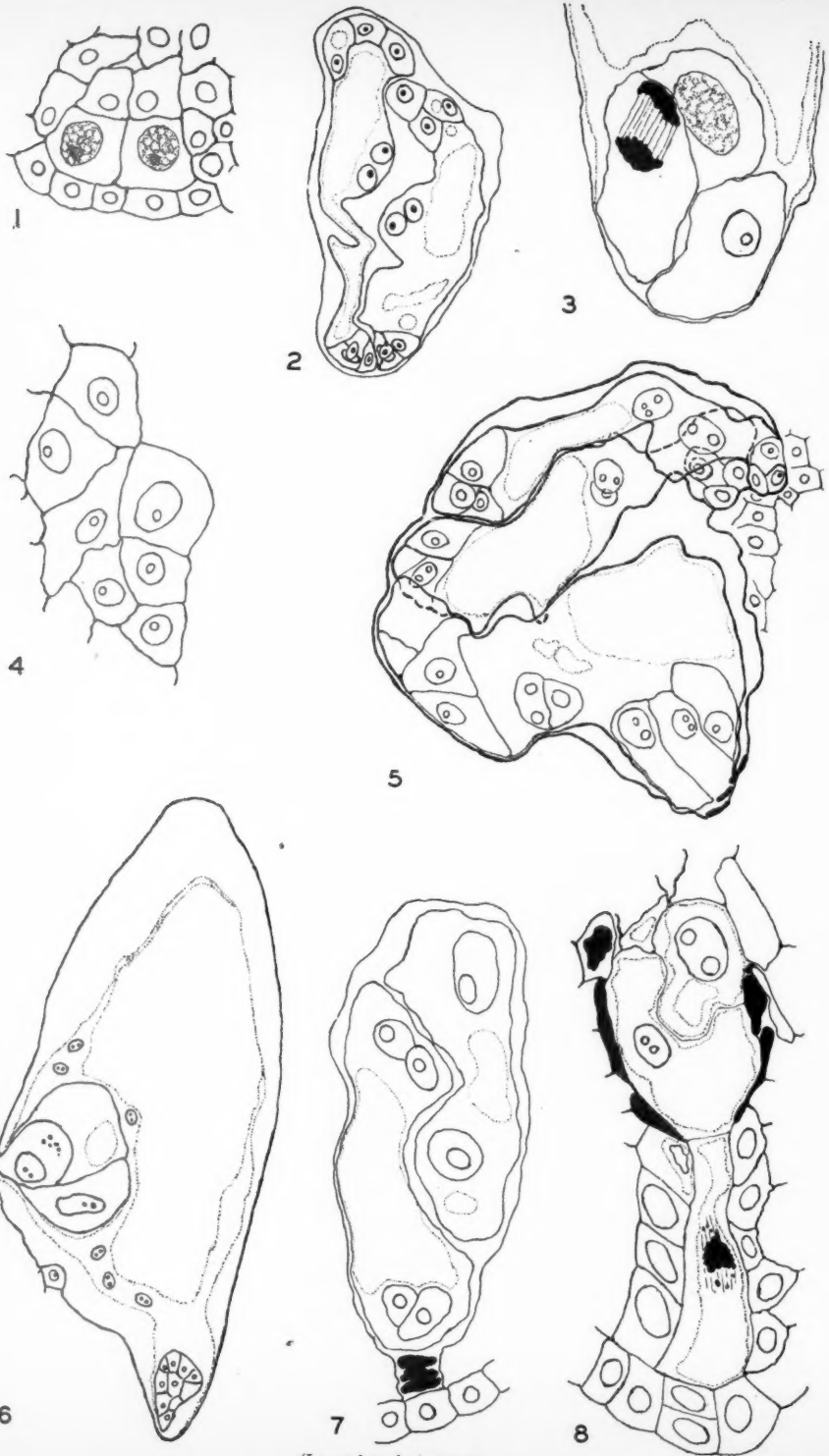
Evidence has been presented elsewhere (9) to show that the reduction divisions take place and that four macrospores result therefrom, the chalazal one eventually developing into the embryo sac. There is evidence that two members of the egg apparatus may sometimes develop, resulting in a haploid-diploid embryo relation.[2] This is in accord with the data given in the companion paper (10) and also with that of earlier workers who have reported haploid-diploid relations in *P. pratensis* twins. There is no evidence that the egg apparatus shown in figure 3 represents this particular combination, since no single embryo sacs were observed with two proembryos or embryos wherein cells with analyzable mitotic-division figures could be found. It appears likely, however, that such was the origin of the haploid-diploid twin set described in some detail elsewhere (10). Although this is true, it remains possible that this twin relation may have arisen from two independent embryo sacs. Each of these would of necessity carry the reduced chromosome complement; one would become diploid through gametic union, whereas the second would develop parthenogenetically because of the stimulative effect of fertilization in the mate.

A second interpretation of figure 3 would indicate the simultaneous development of an egg and a synergid of an embryo sac arising through somatic aposporv. If such were the origin, the resulting plants would be expected to be either diploid-diploid or diploid-triploid. The former combination would be the result of the parthenogenetic development of the two cells in question, and the plants should be maternal in appearance and in progeny behavior. The second possible combination, diploid-triploid, would have to involve the fertilization of one member of the egg apparatus and the parthenogenetic development of the other. The resulting set of twins would probably be dissimilar, with the progenies of both members mostly constant.

DEVELOPMENT FROM A MACROSPORE MOTHER CELL AND FROM A SOMATIC CELL OF THE NUCELLUS.—Enlarged, densely cytoplasmic, nucellar cells frequently are observed at the time of the meiotic divisions. Usually, the macrospore mother cell, or its daughter cells, shows definite indications of collapse when such enlarged somatic cells are conspicuous. This is not always the case, however. Instances have been observed where the macrospore mother cells, or the chalazal macrospore, show no indication of necrosis (fig. 7). It is usually in florets wherein the asexual and sexual embryo sacs, or their counterparts, are developing in an essentially synchronous manner that both members appear functional. Twin embryos, one from each embryo sac, would probably develop into phenotypically different mature plants. Obviously, there is great likelihood that they will be different genotypically because of the apparent heterozygosity of the species. They would be either diploid-diploid or diploid-triploid, depending upon whether the egg of the somatic aposporous embryo sac developed parthenogenetically or as the result of gametic union. The progeny from the twin that traces back to the somatic cell, if aposporous reproduction continues, would be expected to be essentially uniform and, if diploid, maternal in appearance. That of the other twin, from the fertilized, reduced embryo-sac egg, would likely differ phenotypically and in breeding behavior from the maternal parent.

It is desirable to indicate another pos-

[2] Relations refer to the total complements, not to *n*-numbers.



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sible combination from this type of multiple embryo-sac development, for the egg cell with the reduced number might develop parthenogenetically as a result of the stimulative effect of the fertilization of the egg nucleus that arose through somatic aposporous processes. Plants produced from embryos that arose in this manner would be expected to be haploid-triploid, a combination that has not been found. Progenies developed from seed of the haploid, if fertile, would perhaps show considerable segregation. The plant from the triploid could be essentially like the maternal parent in breeding behavior but might be quite different phenotypically.

DEVELOPMENT FROM SEVERAL SOMATIC CELLS.—It is not uncommon to observe, in ovules of Kentucky bluegrass, two or more enlarged nucellar cells in close proximity to the macrospore mother cell which is undergoing division (fig. 8). Usually, when such a condition occurs, there are readily recognizable signs of collapse of the macrospore mother cell, as the figure illustrates. In other cases, however, no evidence of necrosis was evident prior to mitosis in the outermost daughter cell. Frequently, if not usually, the division of the innermost daughter cell does not proceed beyond metaphase or early anaphase. No instances were observed that could be definitely interpreted as a macrospore and two or more somatic cells forming functional embryo sacs. Nevertheless, it is possible that such a development might occur.

The phenotypic characteristics of the mature plants developed from multiple embryos from somatic cells would depend largely upon whether or not fertilization of the egg(s) occurred. If such was not the case, the resulting plants would probably be diploid-diploid and similar to the parent. Conversely, if one of the eggs was fertilized and the second developed parthenogenetically, the twins would be triploid-diploid and in all likelihood dissimilar, with the diploid one maternal in appearance.

A third alternative should be recognized. It is possible that both, or even more, of the several egg cells could be fertilized by male gametes carrying diverse factors. If this occurred, it would be logical to expect that the twins, or other multiple embryo combinations, would be dissimilar in morphological characteristics and be triploid-triploid.

SUPERNUMERARY EMBRYO SACS.—The data in the preceding paragraphs pertain to readily classifiable types of plural "macrogametophytes," such as are commonly encountered in *P. pratensis*. In addition to these several distinct classes of multiple embryo sacs of a single ovule, other types of embryo sacs frequently develop. These appear to be secondary. It was not uncommon to observe these secondary embryo sacs, some of which are incomplete and essentially similar to those discussed and figured by HÅKANSSON (5). These structures consist of two or three antipodals and either one or two polar nuclei. Generally, these incomplete embryo sacs taper gradually toward the position generally occupied by the egg apparatus, which is missing in the defective embryo sacs. Here it flares out some-

FIGS. 1–8.—Origin of multiple macrogametophytes in *Poa pratensis:* Fig. 1, two macrospore mother cells. Fig. 2, two embryo sacs. Fig. 3, egg apparatus showing dividing synergid nucleus and egg nucleus at prophase. Fig. 4, enlarged drawing of bud cell shown in figure 6. Fig. 5, three embryo sacs, one proximal at micropyle, others distal. Fig. 6, longitudinal section of ovule showing nucellar bud cell projecting into lumen. Fig. 7, two embryo sacs, four-nucleate one probably developing from macrospore and two-nucleate one from nucellar cell. Fig. 8, collapsing macrospore mother cell with two enlarged nucellar cells.

what and abuts upon a protruding base of several cells. Sometimes, however, these secondary embryo sacs are complete, i.e., containing three antipodals, two polar nuclei, and a regular three-celled egg apparatus.

Although extensive material has been examined in an attempt to complete the gaps in the data at hand, the developmental sequence remains incomplete. It appears desirable, therefore, to record the observations that are fairly definite. Densely cytoplasmic nucellar cells surrounding the gametophytic cavity have been observed in the region adjacent to the confluence of the antipodals and the vascular strands, between the antipodals and the micropyle, and also on the opposite side of the lumen. These regions, together with the antipodals proper, are the loci from which secondary embryo sacs commonly arise. In several ovules, a particularly densely staining cytoplasmic cell with an enlarged nucleus was observed to protrude into the gametophytic cavity (figs. 4, 6). Intervening stages between this and the mature embryo sacs shown in figure 5 have not been observed. Multiple embryo sacs such as those illustrated in the latter figure have been observed commonly. It seems probable that they may explain some of the instances in *P. pratensis* wherein proembryos and embryos are located proximally and even distally to the micropyle in mature seeds.

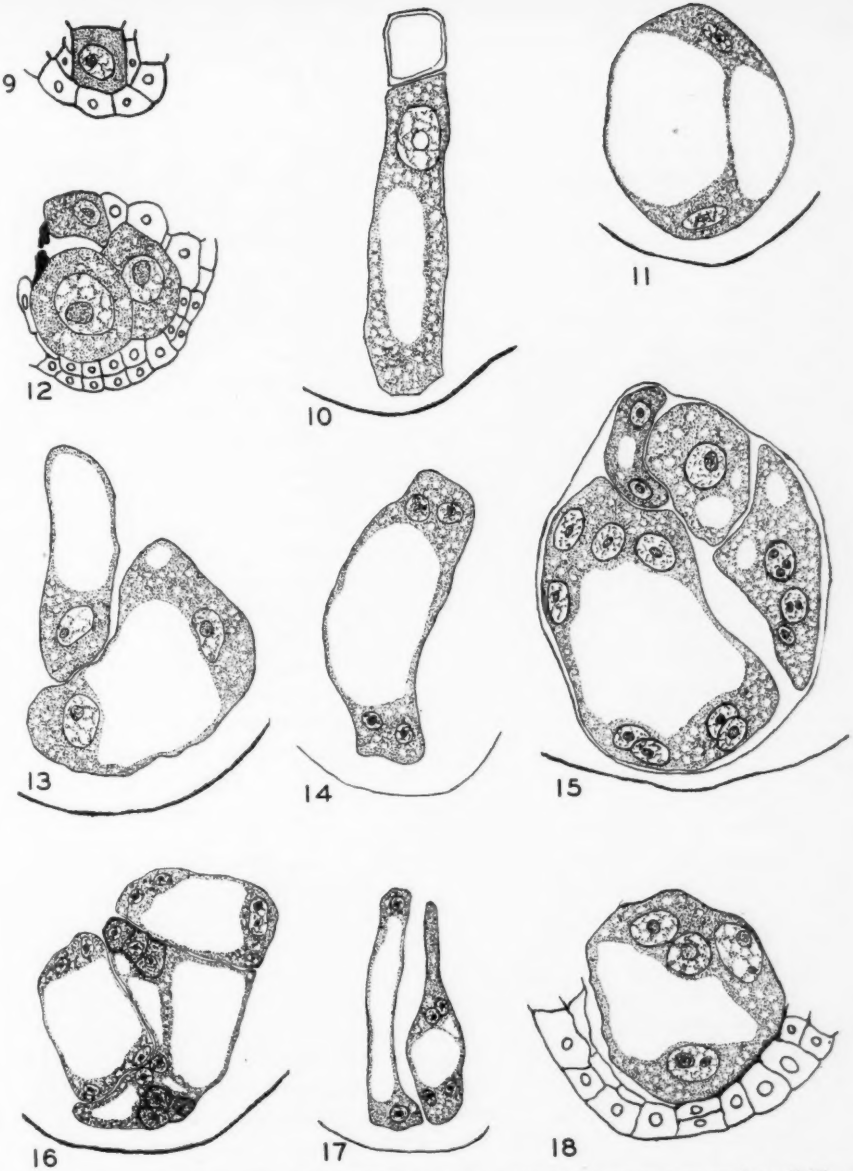
MACROSPOROGENESIS IN THE HAPLOID.—Since earlier studies concerning macrosporogenesis in *P. pratensis* have been limited primarily to biotypes of higher chromosome number, it was considered desirable to examine some of the material of the haploid plant isolated. In none of the limited number of sections available for study were clear nuclear divisions found. Hence it must be assumed from the study of the microsporocytes that the *n*-number equaled 14.

The macrospore mother cell, although smaller in size than those reported elsewhere for diploid plants (9), was similar in shape and in its position under one hypodermal layer of the ovule primordium (fig. 9).

The type of cell with the single large nucleus as shown in figure 10 had not been observed previously. An "empty" cell was located adjacent to the chalazal end of the cell. Usually, the embryo sac is binucleate before a large vacuole of the type shown in this figure appears. The embryo sacs shown in figures 11 and 14 are relatively typical of those occurring in other Kentucky bluegrass ovules containing one embryo sac. Figure 18 is of an atypical embryo sac with a 3 to 1 distribution of the nuclei.

It is uncertain if the dark masses on the left of figure 12 are the remains of the daughter cells of the macrospore mother cell. The three uninucleate cells shown were no doubt derived from the nucellus, since they are mostly located under two tiers of cells of the latter. Figure 13 shows another of the atypical uninucleate-univacuolate cells and a second rather typical binucleate embryo sac. The eight-nucleate embryo sac of figure 15 is rather typical, as is the binucleate one. The uninucleate cell is univacuolate, and the three-nucleate one atypical. The nuclei of the latter differ in size with an accordingly increased number of nucleoli of from one to three. It differs from the other three members of the quadruplet embryo sacs in this respect.

Only the four-nucleate one of the three embryo sacs shown in figure 16 is fairly typical. The upper member contains a giant nucleus. The number and grouping of the cells and nuclei are rather characteristic of Kentucky bluegrass in the



FIGS. 9–18.—Macrosporogenesis in haploid *Poa pratensis* plant: Fig. 9, macrospore mother cell. Fig. 10, elongated, densely cytoplasmic cell in normal position of macrospore mother cell. Fig. 11, two-nucleate developing embryo sac. Fig. 12, three enlarged, densely cytoplasmic, nucellar cells. Fig. 13, two embryo sacs developing, one uninucleate, the other binucleate. Fig. 14, four-nucleate developing embryo sac. Fig. 15, one-, two-, three-, and eight-nucleate developing embryo sacs. Fig. 16, three-nucleate, four-nucleate, and mature embryo sac. Fig. 17, two- and four-nucleate developing embryo sacs. Fig. 18, four-nucleate developing embryo sac.

remaining mature embryo sac. The configuration is, however, quite abnormal.

The two-nucleate macrogametophyte of figure 17 is quite representative of those in other plants of *P. pratensis* examined. The type of the bottle-shaped member on the right had not been seen before.

It is evident that macrosporogenesis can hardly be considered normal for the haploid of the species. Although this is true, some of the developing macrogametophytes examined did not appear to be strikingly different in appearance from those of known fertile apomicts. Misshapen and odd-appearing embryo sacs that are apparently functional occur commonly in the species. The sterility of this plant was perhaps due to genetic factors rather than to the more obvious cytological disturbances.

Discussion

The observations reported in this paper appear baffling when considered without the supplementary data derived from progeny studies from twin and triplet plants of the species (**10**). They give an impression of indeterminancy. They become less confusing, however, when they are considered in light of the resulting mature plants and their progenies. When so considered, they aid materially in explaining the great morphological variation and chromosomal diversity that prevails in the species. It is this relationship that will be considered in the discussion rather than the more limited variations in macrosporogenesis. These will, however, serve as the basis of discussion for each of the points considered.

When sexual lines of the species are isolated, selected plants from their progenies will, for the most part, continue to yield highly, or even completely, diverse progenies. Exceptions occur wherein constant phenotypic appearance in the subsequent progenies is due to genetic changes in the genome (**9**). The high incidence of sterility found in many sexual plants of the species affords a mechanism for the elimination of numerous types. When factors or tendencies for apomixis are present, and where sterility would otherwise ensue, the morphological type is maintained through asexual reproduction. Numerous intermediate plants with tendencies toward both types of reproduction also occur. It is with this intermediate class that the data of this paper are concerned primarily.

It is thought from the material studied that apparently viable and plural embryos should develop essentially synchronously. In ovules where two or more embryo sacs commence development at about the same time, it would seem that the zygote most favorably located with respect to nutrient flow would develop at the expense of the other member or members. It is possible also that earlier initiated members may establish an advantage at the expense of other individuals in the same ovule. This interpretation would explain, in part, the existence of some of the numerous undifferentiated proembryos that are observed in mature or nearly mature seeds. Others might be ascribed to the presence of "supernumerary embryo sacs." These proembryos and partially differentiated embryos may not develop to such an extent that they would be noticeable in germinating seeds.

If the above deductions are valid, it follows that embryo sacs, or their initials, that are located somewhat equally with respect to the nutrient supply and develop in an essentially contemporary manner, would constitute the majority of the cases that develop plural embryos evident at germination. It has been shown that both types of embryo sacs—i.e.,

those that arise from macrospores (9) and those that arise from nucellar cells (9, 13, and others)—may be functional. This would offer a reasonable explanation of the marked polymorphism that prevails in the species.

The divergence in gross morphological features of the progeny of a single sexual plant of *P. pratensis* may be considerable. Some workers have suggested that the species is of hybrid origin; this appears quite probable (9). SMITH, NIELSEN, and AHLGREN (12), after studying several thousands of plants, concluded that about 16% of all the plants studied were aberrants. The most plausible explanation of this condition would seem to be the recombination of genetic factors due to sexual processes, the accumulation of factors due to the fertilization of asexually developed eggs by reduced male gametes, and internal chromosomal and/or genetic changes.

Much evidence has been presented during the last fifteen years to indicate and to support the concept of maintenance by apomixis of the morphological types of Kentucky bluegrass established through the indicated processes. The maintenance of the synthesized types occurs in about 84% of the total population of the species (12). This represents the essentially stable apomictic but highly polymorphic complex of biotypes generally evident in the species. New combinations are being repeatedly formed in approximately 16% of the plants of the species. Some of these may become established. They may arise by any one of the processes indicated. Principally the aberrants may arise by the fertilization of eggs having the reduced number of chromosomes or by the fertilization of unreduced eggs produced as a result of somatic apospory. The formation of haploids as a result of the development of an unfertilized member of the egg apparatus into an embryo and the formation of tetraploids through the somatic doubling of the dividing zygote may also occur. Where these are fertile, or at least partially fertile, they also serve as new points from which polyploid series of apomictic and sexual types may arise. In view of the facts concerning progeny behavior from twin and triplet plants, and the presumed modes of origin of macrogametophytes from which these arose, it is to be expected that numerous biotypes, divergent both in morphology and in chromosome number, will occur.

Summary

The origin of multiple macrogametophytes in *Poa pratensis* was studied and the results presented in relation to known breeding behavior of plants from both plural and single embryos.

Eggs developed following meiosis may result in haploid-diploid and diploid-diploid relationships in the plural embryos and in the mature plants. The origin of these plural embryos is (1) from two members of the egg apparatus and (2) from two macrospore mother cells.

Embryo sacs developed from somatic or nucellar cells may form diploid-diploid, diploid-triploid, and diploid-tetraploid combinations of plural embryos and mature plants. The diploid-diploid combinations are considered to arise from two somatic egg cells or from an egg and a synergid of a single embryo sac that develop parthenogenetically. The diploid-triploid combination probably arises in a similar manner; the egg of one embryo sac develops parthenogenetically, whereas that of the second develops as a result of fertilization by a male gamete carrying approximately the reduced chromosome number. Triploid-triploid combinations could form by the

fertilizing of two cells (two eggs or an egg and a synergid). The diploid member of diploid-tetraploid plural embryos might originate in the manner indicated previously for other combinations, the tetraploid by the parthenogenetic development of the egg with a somatic doubling of the chromosomes of the nuclear complement at the time of the zygotic division, or fertilization with an unreduced or restitution male nucleus, or with a high polyploid gamete. The chromosome complement of the male gamete would have to equal that of the egg nucleus. It is considered quite improbable that origin of tetraploids involving gametes would be of common occurrence.

Plural embryos were found to arise from reduced macrospores and nucellar cells in the same ovule. The embryos resulting from these would be diploid-diploid or diploid-triploid, depending upon whether fertilization of only the egg of the reduced macrogametophyte or fertilization of all of the several members, reduced and unreduced, occurred.

"Supernumerary embryo sacs" might develop from nucellar "bud" cells of the wall of the gametophytic cavity. These embryo sacs may be imperfect and nonfunctional, or they may be complete and account for some of the instances wherein proembryos or well-developed embryos are located at various positions other than at or near the micropylar portion of the seed. The observations are incomplete for such a manner of origin of plural embryos.

Macrosporogenesis in the haploid plant was quite similar to that reported for the higher polyploids of the species. The sterility of this plant is undoubtedly due to genetic rather than to evident cytologic disturbances.

DEPARTMENT OF AGRONOMY
UNIVERSITY OF WISCONSIN
MADISON, WISCONSIN

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ON CARDIOCARPON AND SOME ASSOCIATED PLANT FRAGMENTS FROM IOWA COAL FIELDS

FREDDA D. REED

Introduction

The genus *Cardiocarpon* was established in 1828 by BRONGNIART (3) for some carboniferous seeds which he diagnosed as "fruits comprimés lenticularis, cordiformis ou réniformes terminés par un pointe peu aigüe." Subsequent attempts have been made to break up the genus by employing the term *Cordaicarpon* for seeds known to be related to *Cordaites* and by including in the genus *Samariopsis* those forms in which the wing of the sarcotesta is particularly wide and membranaceous (19). However, since most of the seeds that have been found either are isolated and unattached to vegetative organs and in varying degrees of preservation or are in the form of casts and impressions, there is a consequent lack of constant and definite diagnostic features. Therefore, until more information is available, *Cardiocarpon* remains, and is herein regarded, as a provisional generic term for the reception of carboniferous seeds which are platyspermic with an acute apex and a cordate or sometimes reniform base.

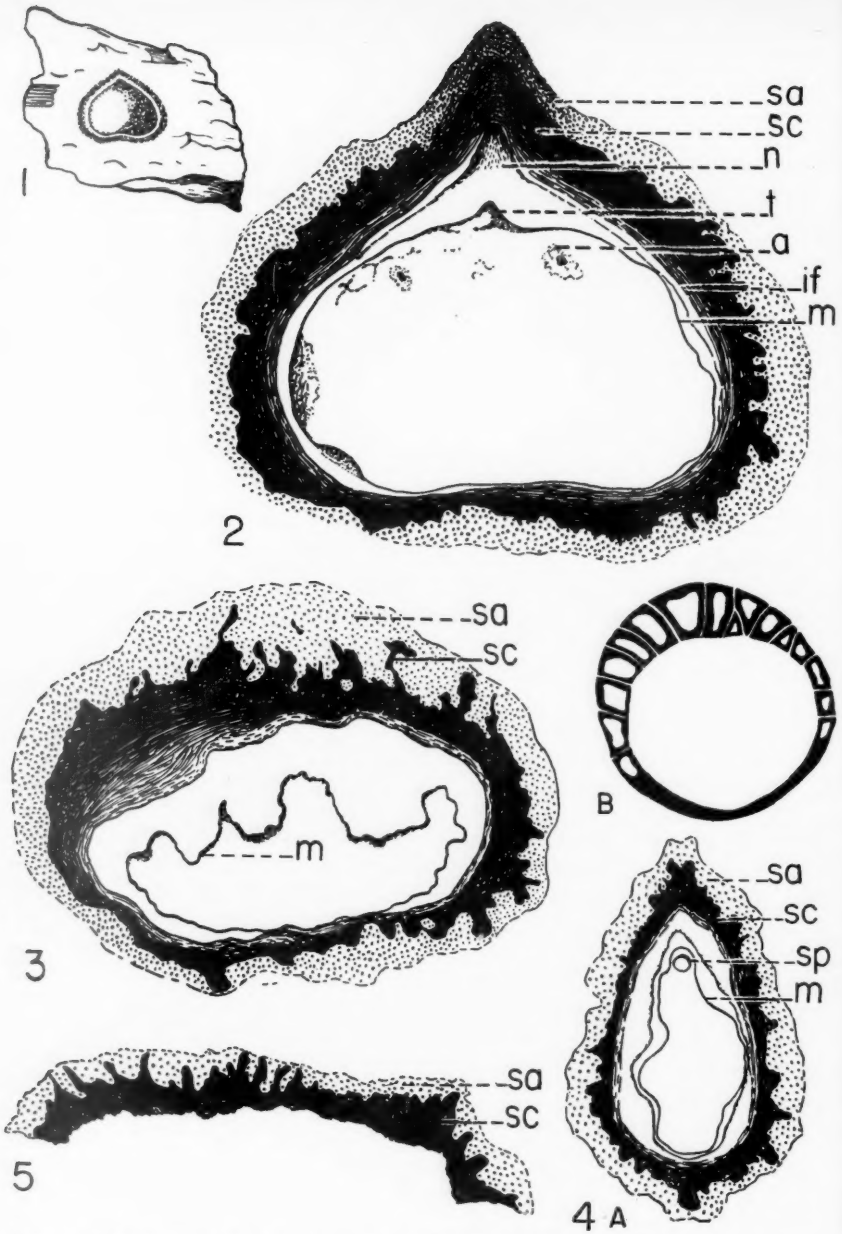
More than seventy species of *Cardiocarpon* have been collected from American coal fields and figured and described by NEWBERRY (14, 15, 16), LESQUEREUX (12, 13), DAWSON (7, 8, 9, 10), ANDREWS (1), WHITE (21, 22), STOPES (20), NOÉ (17), GRAHAM (11), and DARRAH (5, 6). They have been found in geological strata ranging from the Upper Devonian to the Permian, and geographically throughout the range of carboniferous plants in North America. While reinvestigation would doubtless reduce the number of recorded species, there is significance, nevertheless, in their frequency and in their wide geological and geographical range in that both factors indicate for *Cardiocarpon*-bearing plants an important, if not a dominant, place in paleozoic floras.

Material and methods

This investigation is based upon two collections of *Cardiocarpon* from different localities of Iowa. The collections were similar in their mode of preservation in that the seeds were imbedded in calcareous shale. Moreover, the matrix was of such nature that a blow with the rock hammer would shatter it along the cleavage planes into thin flattish pieces. The cleavage planes almost invariably coincide with the flat side of the seed (fig. 1). By so shattering the rocks not only were the seeds revealed but the rock was rendered into pieces of workable sizes without having to section it.

In some instances the seeds were dissected out, coated with cedar oil, which provides a good reflecting surface, and examined for any superficial markings by means of reflected light. The internal structure was determined by grinding the specimens, and at successive levels the surface was polished and the cellular detail was recorded in a series of camera-lucida drawings.

Other specimens were left *in situ*, and the surrounding matrix was ground and polished with the seed. This latter method, although slower, had two dis-



FIGS. 1–5.—*Cardiocarpon*. Fig. 1, impression of *Cardiocarpon* in shale. About natural size. Fig. 2, almost median section of *Cardiocarpon: sa*, sarcotesta; *sc*, sclerotesta; *if*, inner fleshy layer; *n*, nucellus; *t*, tent pole of endosperm; *m*, megaspore membrane; *a*, archegonia. Figs. 3, 4, tangential and transverse sections showing irregular development of sclerotesta. Fig. 4*A*, median longitudinal section showing fern sporangium within megaspore membrane: *sp*, sporangium. Fig. 4*B*, sporangium in detail.

tinct advantages: first, any organically attached structures were preserved and salvaged, and, second, such preparations revealed the structure of other intermingled and associated plant fragments. The latter are interesting of themselves and often furnish clues for the interpretation of the particular specimen that is under investigation.

Investigation

COLLECTION I.—These seeds were collected by HAROLD ALEXANDER, whose account of their origin is as follows: "These specimens are from the rock dump at the mine of the Iowa-Nebraska Coal Co., southwest of Lucas, Iowa. This is located in S.W. ¼ of section 23, Jackson township, in the southwestern portion of Lucas County. This mine was closed in 1925 and the workings are now full of water . . . . this mine worked the 'lower coal' which lies within the Cherokee group of the Pennsylvanian system. . . . It is interesting to note that these specimens are from a locality only about twenty miles removed from where the first petrified *Lepidostrobus* was found."

Altogether there were about thirty specimens of *Cardiocarpon* in this collection. Some of them are little more than casts of the endosperm, whereas others, with their integuments more or less intact, are imbedded in calcareous shale and are intermingled with such other plant particles as fragments of wood, leaves, rootlets, sporangia, and spores.

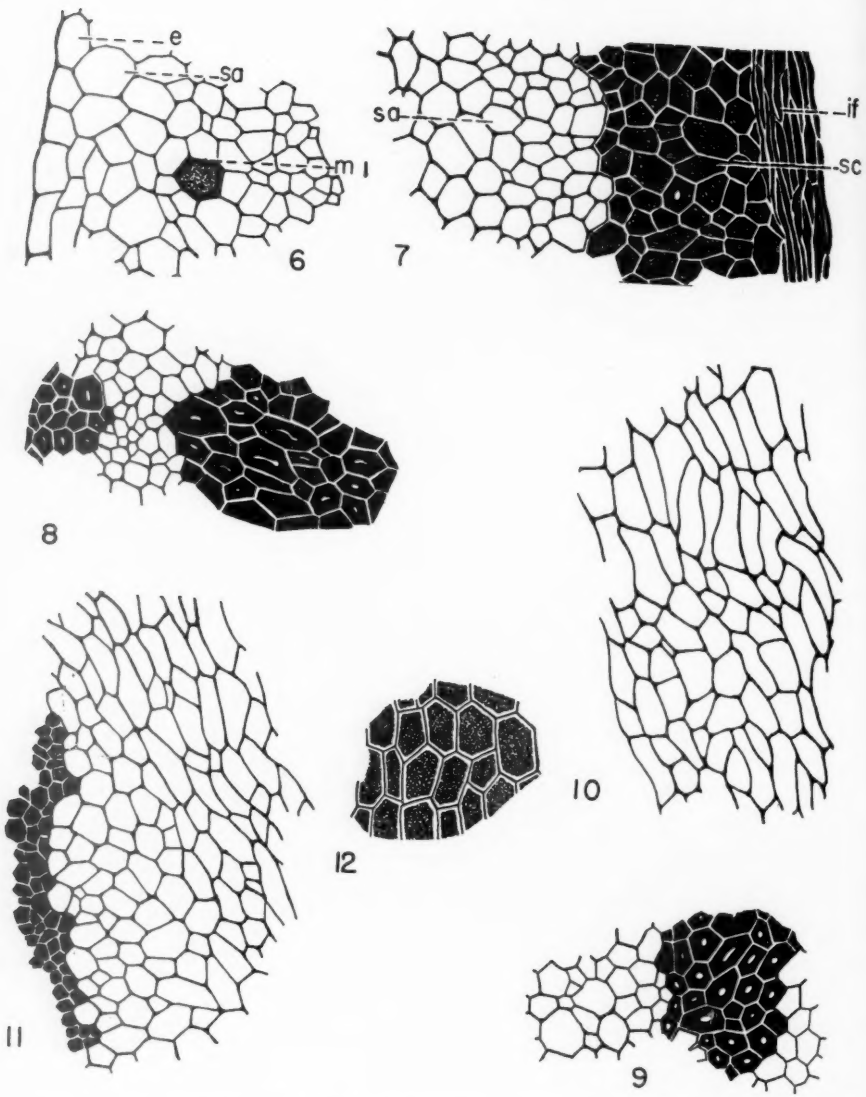
These platyspermic seeds are bilaterally symmetrical. The two planes of symmetry have been designated by Mrs. ARBER (**2**) as the "primary plane," which is the broad plane, and the "secondary plane," which is at right angles to the broad plane. The specimens show some variation in size; the dimensions, excluding the integument, may range from 7 to 10 mm. broad, from 2.5 to 5 mm. thick, and from 5 to 10 mm. long. The integument as seen in any plane is apparently a uniform layer increasing all dimensions by about 1 mm.

Sections of the seed reveal three distinct layers of the integument: the outer fleshy layer, or the sarcotesta; the stony layer, or sclerotesta; and an inner, less well-defined layer of elongated cells here referred to as the "inner fleshy layer."

The sarcotesta is not preserved in its entirety. For the most part the outer limiting cells have disappeared, leaving no hint as to the actual extent of the layer; occasionally, however, the epidermis, or what appears to be epidermis is encountered (fig. 6). Figures 7, 8, 9, 10, and 11 show details of the sarcotesta at different levels and in different planes. The cells are relatively thin-walled and isodiametric; those abutting the sclerotesta are smaller in diameter, and from this region to the periphery they become increasingly larger. Large cells with dark content, as the one shown at *m* in figure 6, are scattered sparingly and at random throughout the sarcotesta and appear to be mucilage bearing. Toward the micropylar region the cells become thicker walled, progressively elongated, and seem to focalize at the apex (figs. 10, 11).

The sclerotesta consists of exceedingly thick-walled, roughly isodiametric cells (figs. 7, 8, 9, 11). In common with some other described specimens of *Cardiocarpon* (**11, 5**), the stony layer is irregularly developed, with the irregularities forming spiny projections extending into the sarcotesta (figs. 2, 3, 4*A*, 5).

The inner fleshy layer is the most difficult to interpret, for the limitations of the cell walls are obscure. Moreover, in this zone it is not always possible to distinguish between the cells of the inner fleshy layer and those of the closely ap-



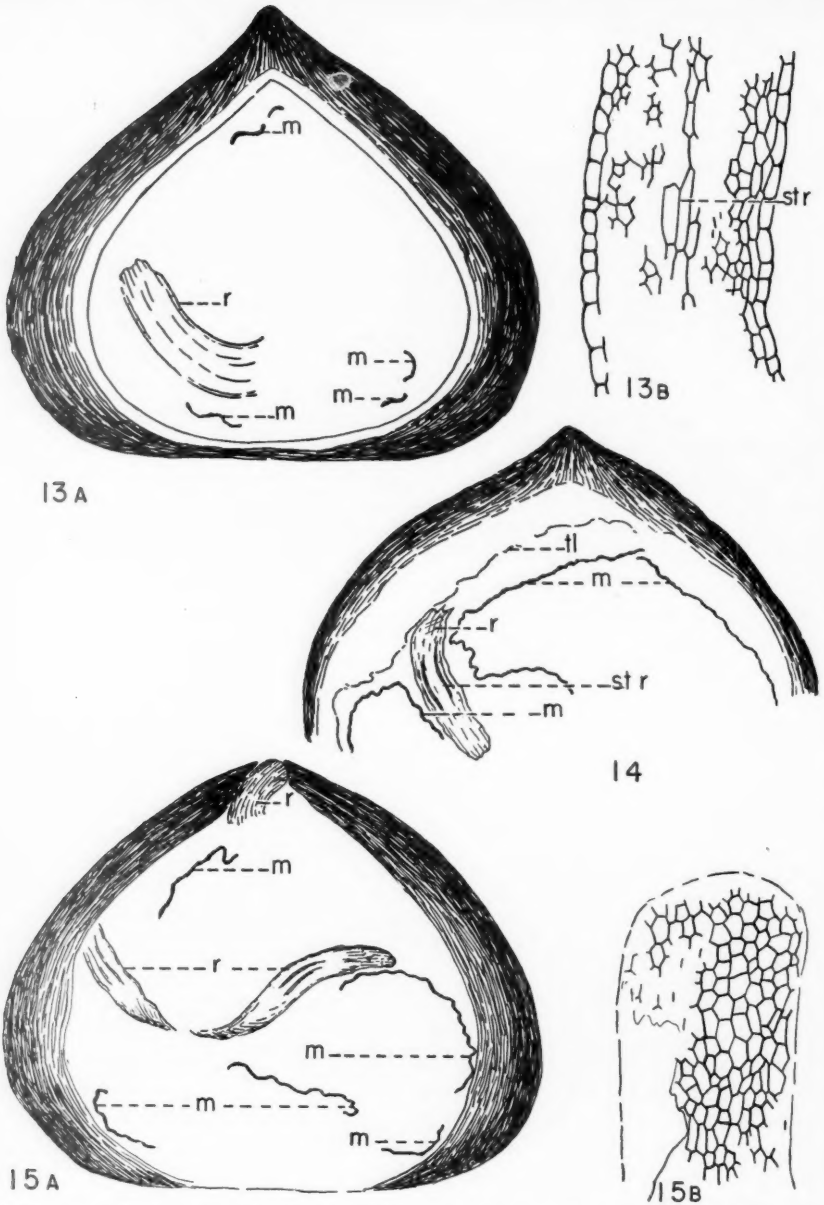
FIGS. 6–12.—*Cardiocarpon*. Fig. 6, portion of sarcotesta: *e*, epidermis; *mi*, mucilage cell. Figs. 7–11, details of integument. Fig. 12, surface view of tapetal cells.

plied nucellus. There is no positive evidence that this is a zone of vascular tissue, yet some of the elongated cells have the appearance of parenchymatous cells of the type that accompany vascular elements. There is also an occasional hint of scalariform markings on some of the walls.

In the genus *Cardiocarpon* the nucellus and integument have been found to diverge near the base of the ovule. In these specimens the course of the nucellus, while it cannot be followed with certainty, does not appear to differ from other members of the group. The tapetal cells form the most conspicuous and characteristic layer of the tissue. Often they are to be seen separated from the rest of the nucellus and in contact with the megaspore membrane. The cells are elongated in the long plane of the ovule and appear to have been filled with some substance that has been preserved as dark-brown material. This material has the same configuration as that of the cells but does not extend to the walls (fig. 12). In these specimens there is no indication of the general structure and nature of the pollen chamber.

As in other paleozoic seeds the megaspore membrane is thick and conspicuous. In a few specimens there has been some shrinkage of the membrane, but in many seeds it appears firm and round and apparently outlines faithfully the shape and extent of the female gametophyte or endosperm. The endosperm as outlined by the megaspore membrane is slightly broader than long and at the micropylar region formed a beak, the "tent pole" of *Ginkgo* and of the Cardiocarpales, extending into the pollen chamber. There are occasional instances of tissue which may be endosperm (fig. 2), but most of the tissue within the membrane is obviously intruded material, as the sporangium in figure 4*A* *sp*, or the rootlet in figures 13*A*, 14, and 15*A*. For the most part the endosperm has been replaced with mineral matter, mostly calcium carbonate, leaving only the faintest remains of the original organic structure.

The seed, which has been figured previously (**18**) and shown here in figure 2, presents an interesting study. The megaspore membrane of this specimen seems intact, and there is no indication of invaded foreign material. The region of the endosperm is almost completely mineralized, with indistinct light-brown markings representing the organic material. Just below and at either side of the tent pole are two more or less defined areas bounded by delicate brown lines and each containing a somewhat dense, centrally located body. The position and size of these areas strongly suggest the central cells of archegonia with the dense bodies as their nuclei. While the markings which represent the organic structure of the endosperm appear to be determined by crystallization rather than by the position of the original cell walls, yet it is possible that the process of crystallization may have been influenced by the density of the protoplasm in which the mineral was being precipitated. Since, by analogy with extant gymnosperms, there would be a decided variation in the density of the central cell and the surrounding cells of the endosperm, it seems highly probable that these bodies replace and, therefore, record the position of the archegonia. Furthermore, that the presence of these areas was not wholly fortuitous is attested by the fact that the same topography was observed in the endosperm of more than one ovule. As was suggested in the former article (**18**), if one assumes a parallel development of the endosperm of *Cardiocarpon* with that of *Ginkgo*, then the two



FIGS. 13–15.—*Cardiocarpon*, series of diagrams showing position of rootlet within region of endosperm: *r*, rootlet; *st r*, stele of rootlet. Figs. 13*B* and 15*B* are details of rootlet from Figs. 13*A* and 15*A*.

dense bodies would occupy the position of the nuclei of the fertilized eggs. Were they the egg nuclei, that is, before fertilization, one would expect to find them at the micropylar end of the central cell rather than centrally located as these appear to be.

Another interesting specimen is the one shown in figures 13*A*, 14, and 15*A*. During the process of grinding down this seed there was observed within the megaspore membrane some plant tissue, as shown in figures 13*B* and 15*B*. The shape and position of this tissue, together with the general aspect of the cells composing it, suggested that it might be embryonic and that it might be either a tangential section through a cotyledon or perhaps through the hypocotyl. From this stage on the grinding proceeded slowly, and the median plane was approached cautiously. Observations and drawings were made at intervals of 10–20μ. When the median plane was reached, the seed was turned over and ground from the opposite side until the "tissue" was visible; then the specimen was mounted.

The two faces of the finished preparation (figs. 13*A*, 15*A*) conclusively demonstrate that the "tissue" is not an embryo or pieces thereof but is an intruded rootlet. Also these two faces not only reveal the nature of the rootlet but indicate its course through the endosperm—a course that is corroborated by the serial drawings made during the progress of the preparation. In figure 15*A* a tangential portion of the rootlet is shown in the region of the nucellus where it had apparently entered from the micropyle; figure 15*B* is a detail of this portion. From this region the rootlet appears to have pursued a downward but winding course. In the plane illustrated in figure 14 there is a rupture in the megaspore membrane, and the rootlet is seen partly within the confines of the endosperm. Again, in figure 13 the portion at *r* is wholly within the endosperm, and its winding or bending course is further indicated. The tortuous position of the rootlet provides both tangential and radial views of it in one plane of the seed. The cellular preservation is so incomplete, however, that there is only a suggestion of its anatomy.

Despite the lack of conclusive evidence there are certain observable facts with regard to this tissue: (*a*) it is one continuous organ from the region of the micropyle through the nucellus and megaspore membrane, into and winding through the endosperm; (*b*) throughout its length a uniform diameter is maintained; (*c*) only primary tissues are apparent, with little evidence of maturation of cells; and (*d*) the incipient vascular elements are apparently central. A summation of these facts seems to establish the identity of this tissue as being that of an intruded rootlet.

If further evidence were needed, it seems amply supplied by the masses of apparently inextricable rootlets of comparable dimensions and anatomy which are sometimes observed in the matrix exterior to the seeds and by the fact that there are other recognizable intruded plant fragments, as the sporangium shown in diagram in figure 4*A* and in detail in figure 4*B*.

COLLECTION II.—The pieces of calcareous shale of this collection were kindly and generously supplied by L. R. WILSON of Coe College. He writes of their source: "The material was collected from the strip mine of the What Cheer Clay Products Company. The Iowa Geological Survey does not seem to have studied the section very thoroughly in the vicinity of What Cheer, Keokuk County. However, its position in the Des Moines Series appears to be rather low."

Of this material, there were dozens of flattish pieces of limestone, each one studded with two or more seeds. In one piece, which was about 3 inches square by $\frac{1}{3}$–$\frac{1}{4}$ inch thick, portions of fifteen different seeds were exposed, and, upon grinding the piece of rock still others were revealed. Intermingled with *Cardiocarpon* were layers of leaves and some stem apices, both of which appear to be of Cordaitean origin, as well as numerous other pieces of roots, stem, sporangia, and spores.

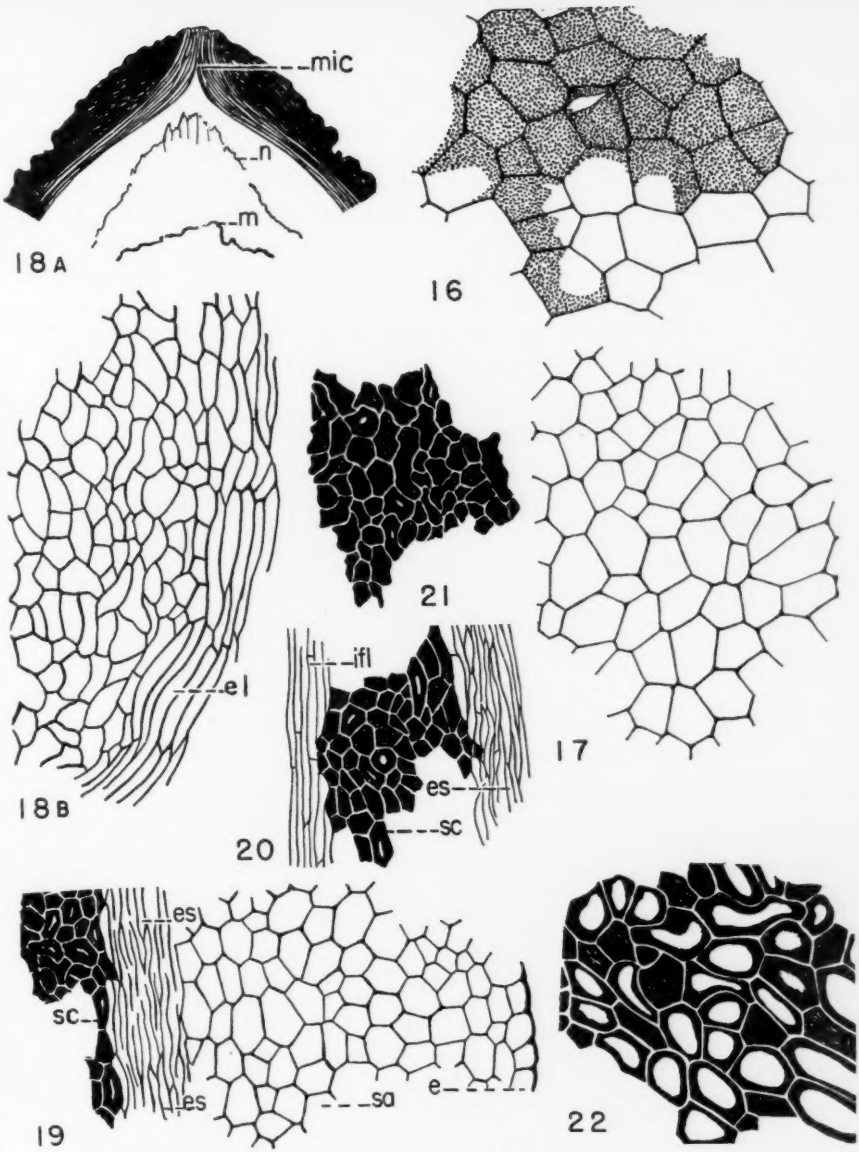
More than a hundred seeds have been examined and have been observed in almost as many planes. In their size, their gross topography, and their internal anatomy, in so far as it could be determined, these specimens bear a striking resemblance to those of the former collection. Only those seeds or such portions of them possessing certain features that make a contribution to our knowledge of the group will be described.

Many specimens were found with the integument apparently intact, and from them it was possible to get good views of the sarcotesta and the characteristics of the several layers of cells composing it. In a surface view the epidermal cells appear golden brown, but in section the material responsible for it is clearly shown to be limited to the outer or superficial walls of the cells, and, therefore, it is thought to represent the cutin (fig. 16). With one exception the remaining layers of the sarcotesta, both in extent and in size of the cells, are similar to the comparable layers of the seeds described above. The exception is in the elongated cells adjacent to the sclerotesta (figs. 19, 20). While no markings were observed on the walls of any of these elongated cells, it is thought that they indicate the presence and position of vascular elements in the outer fleshy layer, as shown in the reconstruction (fig. 26). Moreover, these elongated cells are apparently confined to narrow zones or discrete strands, for in most places the cells of the innermost layers of the sarcotesta are not elongated but are isodiametric (figs. 7, 9).

The sclerotesta (figs. 19, 20, 21, 22), in the size of the cells composing it, in its extent, and in its configuration, is in general aspect conformable with that layer of the former specimens; still, some additional features are revealed because of the more complete preservation. For most of the extent of the sclerotesta the cells are isodiametric, but about the chalazal region and again in the vicinity of the micropyle they are irregular, somewhat elongated, and interlocking. Nor are the cell walls always as thick as is shown in figures 19 and 20, in which the lumen, in section, appears to be reduced to a narrow slit or is almost nonexistent. Occasionally an ovule is encountered in which, throughout the region of the sclerotesta, the walls are much less thick and the lumen is a generous proportion of the cell (fig. 22).

Elongated cells characterize the inner fleshy layer, as was described above (fig. 20). This tissue is demonstrated to be more extensive than was evident in the former specimens, for it not only completely surrounds the nucellus but extends into and lines the micropylar canal (figs. 18*A*, 18*B*).

In a number of instances there is very good evidence of the divergence of the integument and the nucellus, and in each case the pattern conforms to that shown in the reconstruction (fig. 26). Not only is there very little preservation of the nucellar tissue, but the few remaining cells show considerable disintegration, as if the layer had broken down before petrifaction occurred; there is evidence of a



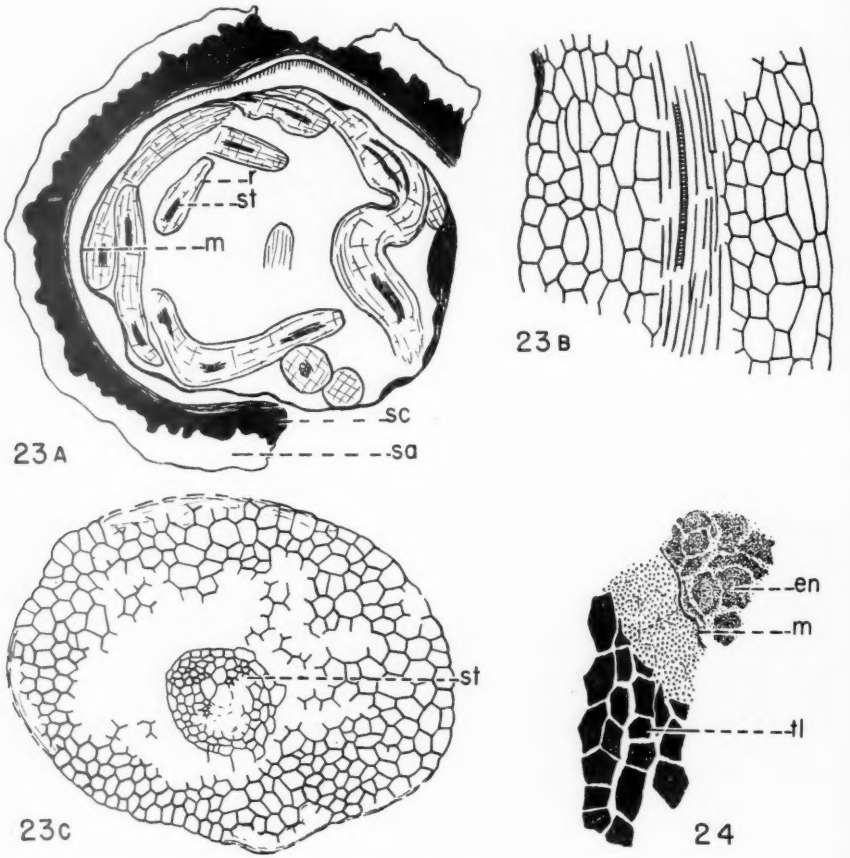
FIGS. 16–22.—*Cardiocarpon*. Fig. 16, surface view of epidermal cells of integument. Fig. 17, tangential section through sarcotesta. Fig. 18*A*, diagram of micropylar region of integument. Fig 18*B*, detail of inner fleshy layer from *A*: *mic*, micropyle; *el*, elongated cells of integument which line micropylar canal. Figs. 19, 20, sections through integument: *es*, elongated cells of sarcotesta; *ifl*, inner fleshy layer. Fig. 22, portion of sclerotesta from seed in which walls of sclerotesta were not so thick.

prominent nucellar beak (figs. 18*A*, 26). The tapetal cells of the nucellus verify the description recorded for that layer from the seeds of collection I.

Almost every specimen examined, whatever the degree of preservation, has some portion of the pronounced megaspore membrane (figs. 23*A*, 25, 26); and in many of them it appears to be intact. Within the megaspore membrane there is no tissue that is manifestly megagametophyte, nor is there any tissue that can be demonstrated as being embryo.

In some instances there is within the megaspore membrane some organic material which is conceivably endosperm. There is some indication of cellular organization of this material, but it is without cell walls and is of such an amorphous nature that no clue is left as to its original structure (fig. 24).

It is both interesting and curious that



FIGS. 23–24.—*Cardiocarpon*. Fig. 23*A*, rootlet in region of endosperm. Fig. 23*B*, detail of rootlet in longitudinal section. Fig. 23*C*, rootlet in transverse section. Fig. 24, portion from tangential section showing indication of cellular organization of endosperm: *en*, endosperm.

in fully half these seeds there were lengths of rootlets which, in their proportions and in their structure, in so far as they are demonstrable, are comparable to the one shown in figures 13, 14, and 15. In some specimens only a few short lengths were visible, whereas in others long pieces of rootlets apparently wound around and back and forth throughout the region of the endosperm (fig. 23*A*). Again, some rootlets are clearly within the megaspore membrane (fig. 23*A*), while others are found inserted between the integument and nucellus. Moreover, in some cases the rootlets seemed to have entered from the micropyle; in others pieces of rootlets are associated with ruptured places in the integument near the chalazal end of the ovule, as if they had made their entrance or exit from that region. Regardless of its position within the ovule or of its fragmentary condition, the rootlet maintains a constant diameter and apparently uniform structure (figs. 23*B*, 23*C*), thereby removing any possibility of its being regarded as an embryo or portion thereof.

Some associated fragments

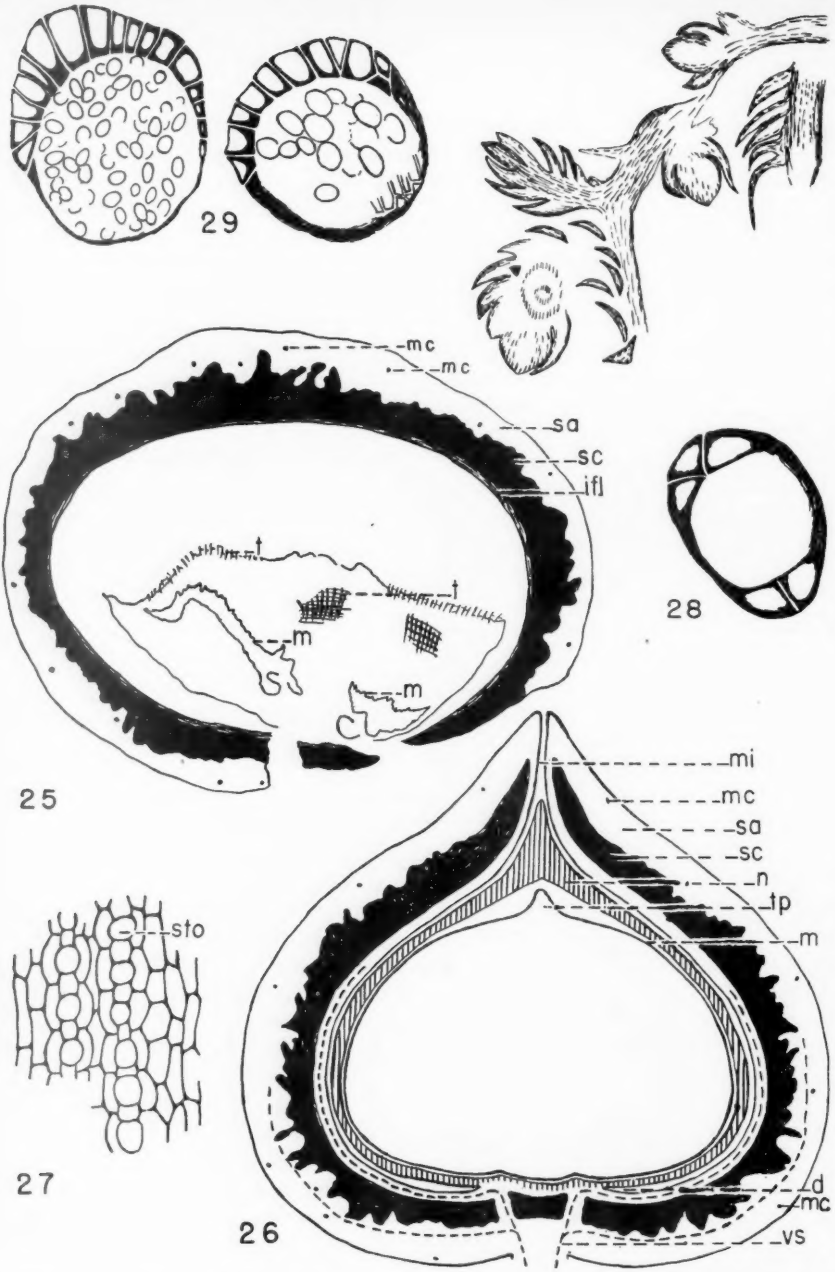
Cordaitean leaves.—Of the associated plant fragments, the most conspicuous and the most abundant were some leaves of *Cordaites*. These were found stratified throughout the pieces of limestone and were arranged layer upon layer. Their size, parallel venation, and the longitudinal strands of sclerotic cells establish these leaves in the genus *Cordaites*, but beyond that the preservation is too meager to admit specific identification. Nevertheless, certain features are worthy of record because of their value in subsequent investigations and also because these leaves may prove to be a link in establishing the identity and structure of the vegetative organs that produced the above-described specimens of *Cardiocarpon*.

The leaves were relatively large. Some of the pieces had a constant width of about 1 inch for 2 or 3 inches in length, and they must then have been much longer. Preservation was limited to the sclerotic and cuticularized cells, and it is the latter that provides the pattern of the epidermis (fig. 27). Areas like the one shown in figure 27 are extremely limited, but they do indicate two characteristics of the epidermis: (*a*) there is a definite and regular arrangement of the stomata and (*b*) in surface view the guard cells are not visible; therefore, the stomata must have been sunken.

Stem apex.—A few stem apices were encountered, one of which is shown in figure 25. No stem tip was found in organic connection with the Cordaitean leaves or with the specimens of *Cardiocarpon*, yet all three—the stem tip, the mature leaves, and the ovules—have certain cell characteristics in common, and it is believed that all may have been part of one plant. As in the case of the leaves there is so little preservation that only the general topography of these stem tips is evident. The closely imbricated arrangement of the small leaves or bracts indicates that these tips were buds. Some buds appear to be strictly vegetative, while interspersed among the appendages or bracts of other tips were microsporangia filled with innumerable microspores.

Discussion

These specimens of *Cardiocarpon* seem to be conformable, in so far as they are preserved, with *Cardiocarpus spinatus* Graham, a species based upon two specimens from Richland County, Illinois (11). Darrah (5) has described some similar seeds from the vicinity of Wau-



FIGS. 25–29.—Figs. 25, 26, *Cardiocarpon*. Fig. 25, proximity and relative size and proportions of *Cardiocarpon* and vegetative tip of *Cordaites*. Fig. 26, reconstruction of median longitudinal section. Fig. 27, portion of epidermis of *Cordaites* leaf: *sto*, stoma. Figs. 28, 29, some associated fern sporangia.

kee, Iowa; and, while he employs the generic term *Cordaicarpus* instead of *Cardiocarpus*, he believes his specimens to belong to the same genus and species as those described by GRAHAM. However, inasmuch as GRAHAM's specimens were incomplete and his specific term was derived from the spiny character of the stony layer of the integument, a character which would not be visible if the seed were entire, it seems questionable whether the term *spinatus* is appropriate. Furthermore, both NEWBERRY (**14, 15, 16**) and LESQUEREUX (**12, 13**) have figured specimens of *Cardiocarpon* which, from the illustrations, are so much like the recently described ones that a comparative study would doubtless provide an already established specific name. The descriptions of the earlier investigators did not include cell structure and anatomy but were based upon superficial characters of size, shape, and markings. By a comparison of such superficial characters, the above-described specimens appear strikingly like *Cardiocarpus affinis* Lesq. Therefore, except for retaining BRONGNIART'S (**3, 4**) generic term *Cardiocarpon*, the same name, *Cardiocarpon affinis* Lesq., is here provisionally applied.

Summary

1. The plant fragments herein described were found in two collections of shaly limestone. Both collections were from the Upper Carboniferous: collection I came from a coal mine near Lucas, Iowa; collection II, from a strip mine in the vicinity of What Cheer, Iowa.

2. Of the plant fragments in both collections, *Cardiocarpon* was the most conspicuous. The description and reconstruction of *Cardiocarpon* are based upon an investigation of more than a hundred different specimens.

3. No specimen of *Cardiocarpon* was found attached to vegetative organs. They are cordate, platyspermic seeds and measure about 1 cm. wide, by 1 cm. long, by 5 mm. thick. There is a single integument of three layers: sarcotesta, sclerotesta, and inner fleshy layer. There is evidence of a double vascular system, one series in the sarcotesta and one in the inner fleshy layer.

4. Some specimens appear to have archegonia. Within the endosperm of many of them there is extraneous and intruded plant tissue.

5. These specimens are apparently conformable with *Cardiocarpon affinis* Lesq., and, as such, have been provisionally designated.

6. The associated fragments are: leaves of *Cordaites;* stem tips with buds, in some of which are found microsporangia, of *Cordaites;* some unidentified rootlets; and some unidentified fern sporangia.

MOUNT HOLYOKE COLLEGE
SOUTH HADLEY, MASSACHUSETTS

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CONTROL OF FLOWER FORMATION AND FRUIT SIZE IN THE PINEAPPLE

J. VAN OVERBEEK[1]

Introduction

Auxins affect, and under certain conditions control, a variety of physiological processes. Among these may be mentioned elongation of stems, formation and growth of roots, development of lateral buds, abscission, water uptake, protoplasmic streaming, and enzymatic processes. In general, one may say that auxins exert their effects on higher plants as a whole rather than on a specific group or species. An exception seems to be flower induction in the pineapple. It has been shown, so far, that only this plant and perhaps some other bromeliads[2] respond to these substances by forming flowers. The possibility exists, however, that auxins have a general role in flower formation, even though this has not been demonstrated as yet. It was therefore felt that a study of flowering in the pineapple might give rise to information applicable to the control of flowering in general.

The fact that auxins do not induce flowering when applied to most plants could be interpreted as a lack of relationship between auxin and flowering. But it has been shown (**11**) that applications of auxins to cuttings are without effect unless they can act co-ordinately with substances produced by the leaves. These substances were later identified as certain sugars and nitrogenous compounds (**13**). In other words, the part auxin plays in floral initiation may be

[1] The assistance of HÉCTOR J. CRUZADO, ELBA SANTIAGO DE VÁZQUEZ, and GUILLERMO DÁVILA is gratefully acknowledged.

[2] *Billbergia* and some other bromeliads respond to ethylene (**4**), as do pineapples (**8, 3**).

masked by the absence of other requisite factors.

FLOWER FORMATION IN THE PINEAPPLE PLANT.—During its vegetative phase of growth, the axis of the pineapple plant remains short, and the apex continues to produce foliage leaves. Without treatments this vegetative apex may change, during the winter months, into one which produces an inflorescence. A description of this process has been given by COOPER (3). When flower formation takes place, the formation of foliage leaves is halted and the axis begins to elongate (fig. 1).

One may distinguish between pineapple varieties of which a large percentage of the plants flower at normal flowering time and others which are characterized by a low percentage of flowering. Among the first the Red Spanish, the principal commercial variety in Puerto Rico, may be named. During the winter season not only all mature plants but also many immature plants flower. The flowering of immature plants is highly undesirable.

The Cabezona is a variety of the opposite type. During the flowering season only a relatively small percentage of the mature plants flower, and rarely is a flower formed on an immature plant. In our experience the Cabezona flowers very infrequently during the first year after planting, about 25% of the plants produce flowers during the second year, and the remaining plants, although of mature size and capable of bearing large fruits, will produce in later seasons. It is not uncommon to find plants which produced their first fruit during their fifth season. This natural tendency to limited flowering makes the Cabezona highly suitable for experiments with flower-inducing substances. Delayed flowering is a liability from the commercial standpoint at present, but this characteristic can be turned into an asset, as will be shown in the discussion.

Methods

The Cabezona, which is the commercial variety of the Lajas Valley, was used for the experiments here reported. Preliminary experiments were carried out at Mayagüez, but the bulk of the work was done at the Ortiz[3] farm at Lajas. There the average rainfall is 29 inches annually, with a dry season occurring during the winter and spring months (5). The plants were 14 months old when the work was begun and 28 months old when the present experiments were terminated.

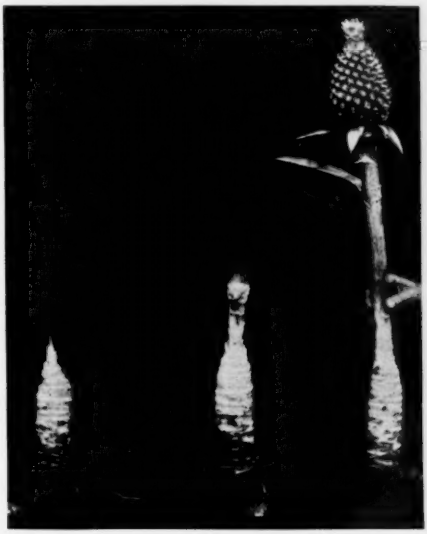

FIG. 1.—Different stages of elongation of axis of Cabezona pineapple. Plants 27 months old. Leaves stripped. *Left*, vegetative stage. *Center*, early (natural) flowering stage, about 7 weeks after flower initiation. *Right*, 3 months and 20 days after treatment with 10 p.p.m. naphthaleneacetic acid (NA). Photo, December 26.

[3] The author is indebted to Dr. A. ORTIZ ROMEU, who generously made part of his best plants available for experimentation.

Applications of flower-inducing substances were made to the center of the plant. Each plant was treated only once. In the early experiments a pressure spray pump was used, and leaves around the center were also sprayed. In later experiments the solutions were poured in the center of the plant with a small beaker. In both instances 50 cc. were applied per plant. The use of Carbowax added to the solutions was found to be of no benefit. Nontreated controls alternated with treated plants in every row.

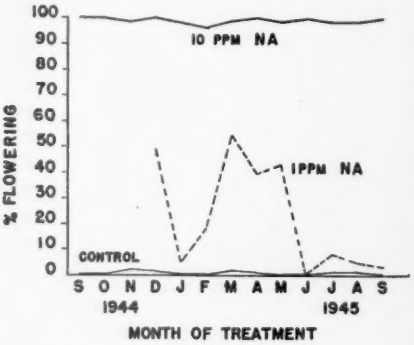

FIG. 2.—Flower induction in Cabezona pineapple throughout year by single treatment with naphthaleneacetic acid (NA). Each plant received 50 cc. of solutions. Plants were 2 years old (after planting) in September, 1945, when last treatment was made. Each point represents average of 40–50 plants.

Naphthaleneacetic acid (NA) was the principal compound used. An aqueous stock solution of 0.1% was carried to the field, where further dilutions were made with distilled water. The acid of this stock solution was neutralized with KOH, NaOH, or NH_4OH in order to prevent the NA from crystallizing. No difference in flower-inducing ability was found between the K, Na, or NH_4 salts of NA, or between the salt and the acid. Another series of experiments was performed using 2,4-dichlorophenoxyacetic acid (2,4-D), neutralized as indicated above, as a flower-inducing agent.

Observations were made once per month, and counts for flowering were made 2 months after treatment. All fruits mentioned were first fruits and not ratoons. When statistical analyses were made, the methods outlined by SNEDECOR (9) were followed.

FIG. 3.—Flower formation in Cabezona pineapple 7 weeks after treatment with 10 p.p.m. NA. At time of treatment plant was 24 months old. Photo, November 12.

Results

RESPONSE TO FLOWER-INDUCING AGENTS THROUGHOUT THE YEAR

When the investigations were begun, no data on flower induction by means of chemical treatments throughout the different seasons were available. From Hawaii a single case was reported in which flowering 4 months ahead of the normal time was obtained (1), and from Florida it was reported that NA treatments given during July, August, and September were not successful, while

those given during October were (2). As has been shown in a preliminary note (10) and in figure 2, NA will induce flowers in every month of the year. Throughout the year the plants responded with great regularity to 10 p.p.m. NA, which caused practically 100% flowering. Flowers became externally visible approximately 7 weeks after treatment (fig. 3), and fruits were ready for harvesting approximately 5 months after treatment (fig. 4).

FIG. 4.—Fruit of Cabezona pineapple at harvest (December 19; out of season) 5 months after treatment (July 21). At harvest, plant was 27 months old, had 40 mature leaves, and fruit weight of 4.7 kg. This plant, and consequently its fruit, is larger than average for its age (see fig. 8 and table 2). Note "slips" below fruit, which are used for propagation.

A concentration of 1 p.p.m. appears to lie just at the threshold of flower induction (fig. 2), while 5 p.p.m. appear to be just as effective as 10 p.p.m. (fig. 5). The greater response to 1 p.p.m. during the period from December to May (fig. 2) is most likely attributable to a period of relative drought and not to changes in sensitivity. During dry weather there is less water in the center of the plant than during periods with frequent rainfall, and hence the applications will be diluted to a lesser degree and there will be less overflow during the dry season.

When applied in quantities of 50 cc., the amount of growth regulator used is, for 1, 5, and 10 p.p.m., respectively, 0.05, 0.25, and 0.5 mg. per plant. Within certain limits it is immaterial whether NA is applied in relatively strong or weak solutions as long as the total amount applied remains the same (table 1).

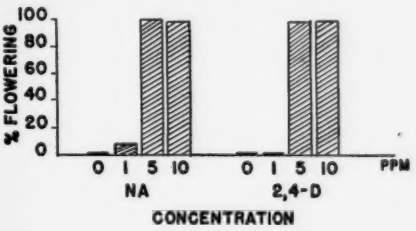

FIG. 5.—Flower induction as function of concentration of NA and 2,4-D, applied by a single treatment in July, when plants were 22 months old. Each value represents average of 40–50 plants.

RELATION BETWEEN NUMBER OF LEAVES AND FRUIT WEIGHT

It is well known that small pineapple plants produce small fruits and large plants large fruits (3). In order to investigate this relation more precisely, a number of experiments were performed in which individual fruits were weighed at harvest time, and, in addition, leaves which were green and over a foot in

TABLE 1

FLOWER FORMATION ON 2-YEAR-OLD CABEZONA PINEAPPLE PLANTS AFTER TREATMENT WITH 0.5 MG. OF NAPHTHALENEACETIC ACID (NA) IN DIFFERENT AMOUNTS OF DILUENT. TREATMENTS ON OCTOBER 5, 1945. OBSERVATIONS ON DECEMBER 19, 1945

Solutions applied	Total mg. NA per plant	No. of plants treated	% flowering
50 cc. of 0.001%.....	0.5	26	81
5 cc. of 0.01%.....	0.5	33	76
3 drops of 1% in alc....	0.5	27	30
None.....	0	88	0*

* Forty days later, on January 29, 1946, 23% of the controls were in flower because the flowering season had arrived.

length on each plant were counted, dead leaves and small ones being omitted. The fruit weight included the weight of the crown.

Figure 6 shows the result of such a test in which five hundred fruit weights were correlated with leaf numbers. Each fruit is represented by a cross. The plants were grown on a dry hillside without irrigation or fertilizer. When 18 months old, they were treated with 0.25 mg. of NA per plant. All flowered, regardless of size, and produced fruits which were harvested at the end of August, about $4\frac{1}{2}$ months after treatment.

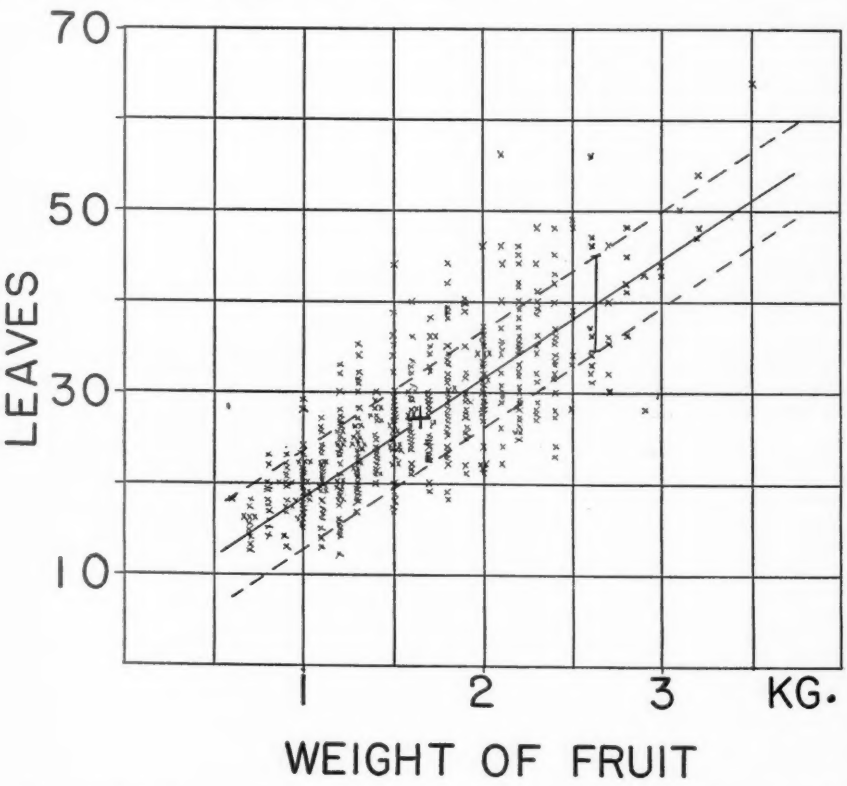

FIG. 6.—Relation between number of leaves per plant and weight of pineapple it produces. On April 15 plants were treated with 5 p.p.m. NA, when they were 18 months old. Harvest took place on August 24. Plants grew under adverse conditions. Slanting solid line is regression line; broken lines on both sides indicate standard error of estimate.

The correlation between leaf number and fruit size is statistically highly significant. A correlation coefficient of 0.76 was found; under the conditions of the experiment only 0.12 is required at the 1% level. The average fruit weight was 1.65 kg. and the average number of leaves 27.4. These values are represented by + in the diagram of figure 6. The regression coefficient was 11.5, which crosses of figure 7 show the relation between leaf number and fruit weight on nontreated plants. The plants were 22 months old when harvested during the middle of May. The circles, on the left, show the relation between leaf number and fruit weight of plants treated with 10 p.p.m. NA on November 25, and which were 20 months old at the time of harvest. The fruits of plants which had

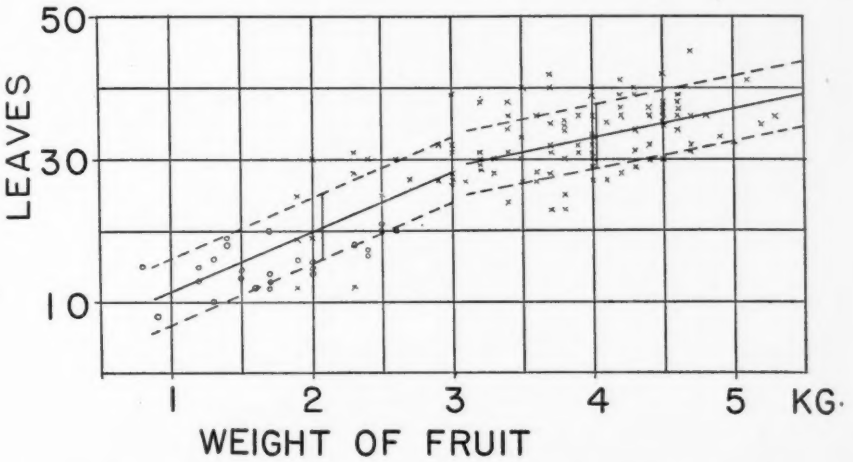

FIG. 7.—Relation between number of leaves per plant and weight of its fruit produced during normal season (crosses). Plants were 22 months old at time of harvest (May 15). Circles on left represent additional data for small plants treated with 10 p.p.m. NA when 14 months old and harvested at same time as naturally produced fruit. Plants grew under good conditions and may be compared with those of fig. 8.

signifies that an increase of 11.5 leaves was accompanied by an increase of 1 kg. in fruit weight. From these data the regression line was constructed, which is represented as the slanting solid line in the diagram. Finally, the standard error of estimate was calculated, the value of which is indicated by the broken lines on each side of the regression line.

Two additional correlation tests are given in figures 7 and 8. The plants of these tests were grown under excellent conditions on land where the plants referred to in figures 2 and 5 grew. The flowered naturally (crosses) represented only 8% of the total number of nontreated plants in that block. They were all large plants. Some additional plants (12%) were flowering at that time and produced fruits later in the season, while the remaining 76% did not flower during this season. Nearly 100% of the young, treated plants (circles) flowered (fig. 2).

Regression lines were constructed for fruits of 3 kg. or less and for fruits of more than 3 kg. The correlation coefficients (0.79 and 0.41, respectively) were statistically highly significant. The

regression lines and the standard error of estimate are shown in the diagrams in the same manner as in figure 6.

The diagram for figure 7 is comparable to that of figure 8 in that plants of the same block were used. They differ in that the plants of figure 8 were all treated with NA and harvested out of season (December 19). Treatment took place on July 21, when the plants were 22 months old. A comparison of figures 7 and 8 shows that NA-induced fruit, which was produced out of season, had exactly the same weight as that produced without treatment during the regular flowering season. For example, in order to produce a 4-kg. fruit during the season, an average number of thirty-two leaves is required (fig. 7). For NA-induced fruit, harvested out of season, we found precisely the same relation (fig. 8). Similarly, an average of twenty-eight leaves will give a fruit of 3 kg., regardless of whether the fruit was produced during the season and without treatment (fig. 7) or out of season and with treatment (fig. 8).

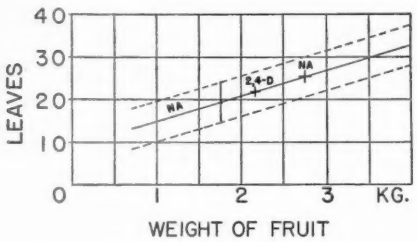

FIG. 8.—Relation between number of leaves per plant and weight of its fruit produced out of season. This diagram represents plants treated with NA (pooled data of 5 and 10 p.p.m.) on July 21, when they were 22 months old. Fruits harvested on December 19 and January 4 (pooled data). Cross marked NA shows average weight and average leaf number of NA-treated plants. Cross marked 2,4-D shows same for plants treated with 2,4-D with same concentrations and at same time as NA plants. Plants grew under good conditions in same block as those of fig. 7. Data of figures 7 and 8 are comparable (further data in table 2).

COMPARISON BETWEEN NA AND 2,4-D TREATMENTS

Previously (10) it was shown that with 2,4-dichlorophenoxyacetic acid (2,4-D) flowers may be induced with equal facility as with NA (fig. 5). Fruit production with 2,4-D is, however, somewhat delayed. For instance, in one test of 96 NA-treated plants 79% were harvested 5 months after treatment. Of 109 comparable plants treated with 2,4-D only, 56% had harvestable fruit 5 months after treatment. The remaining fruits were harvested 3 weeks later. Table 2 summarizes the results of flower induction with NA and 2,4-D. It is of interest to note that the smaller average weight of fruits of the 2,4-D plants, as compared with the NA plants, is accompanied by a proportionately smaller leaf number. This places the point (+) for average fruit weight and leaf number for 2,4-D plants exactly on the regression

TABLE 2

COMPARISON AT HARVEST TIME (DECEMBER 19) OF PINEAPPLE PLANTS OF CABEZONA VARIETY TREATED WITH NAPHTHALENEACETIC ACID (NA) AND 2,4-DICHLOROPHENOXYACETIC ACID (2,4-D). POOLED DATA OF CONCENTRATIONS OF 5 AND 10 P.P.M. TREATMENTS ON JULY 21, 1945

	NA	2,4-D	Difference*
Av. no. of leaves.....	25.2 (100%)	21.8 (86.5%)	3.4 (13.5%)
Av. wt. of fruit (kg.)..	2.78 (100%)	2.18 (78.5%)	0.60 (21.5%)
Av. length of fruit stalk (cm.).....	34.9 (100%)	28.8 (82.5%)	6.1 (17.5%)
Degrees of freedom...	75	105	

* All differences statistically highly significant.

line for NA (fig. 8). This suggests that the lower average weight of 2,4-D fruit is caused by a lower leaf number of the 2,4-D plants as compared with those treated with NA. This, in turn, may be an effect of 2,4-D in preventing some leaves which were young at the time of treatment from reaching maturity. The interference of 2,4-D with normal leaf growth is well known (**14**), and certain relatively tender-leaved varieties of pineapple, such as the Smooth Cayenne, showed leaf deformations after treatments with low quantities of 2,4-D.

Discussion

Theoretical aspects

Which factors, external and internal, are responsible for the fact that under natural conditions flowers are initiated in the pineapple only in the winter season? Since the first flowers of the pineapple appear in December (fig. 1), flower initiation must have taken place early in November. When one examines which *external* factors are different in November as compared with the months preceding it, the decrease in length of day is especially notable. When length of day is plotted against the time of year, one finds for Puerto Rico that from April to August the length of day does not change greatly. For the middle of the month the daylengths are:[4] April, 12^h34^m; May, 12^h59^m; June, 13^h12^m; July, 13^h7^m; August, 12^h45^m. After August the length of day drops relatively sharply: September, 12^h14^m; October, 11^h44^m; November, 11^h17^m. After November it does not drop much farther: December, 11^h3^m; January, 11^h10^m. Then a rise sets in again: February, 11^h34^m; March, 12^h2^m.

October and November, therefore, must be critical months for the initiation of flowers in short-day plants. This is demonstrated by sugar-cane varieties which respond to changes in the length of day (**7**), and which in Puerto Rico always arrow with great regularity in the middle of November.

Preliminary data indicate that the pineapple also may be a short-day plant. Not only does its flowering period coincide with the period of the shortest daylength but it appears that an artificially increased daylength prevents natural flowering (but not chemically induced flowering). This was shown by rigging four 200-watt bulbs above forty-six pineapple plants. These were approaching their third season but had not yet flowered. By means of a time switch the lights were turned on from 7:00 P.M. until midnight. This light treatment was begun in August and was continued until January. After one month of extra illumination five plants were given 10 p.p.m. NA. Control plants, not receiving additional light, were treated similarly. Twenty days later five additional plants under the lights and five controls received 10 p.p.m. NA. Two months after the NA had been applied the plants receiving extra light flowered as well as the controls which had not received extra illumination. A long day, therefore, cannot prevent naphthaleneacetic acid from inducing flowers in the pineapple plant.

The same experiment yielded additional information. In February, well within the normal flowering season, 41% of the 223 control plants (not illuminated or treated chemically) had formed flowers. On the other hand, none of the 36 illuminated but nonchemically treated plants had then flowered. This suggests that the pineapple plant is a short-day plant. Further experiments are necessary, however, to ascertain this. It has

[4] Meteorological data obtained through courtesy of Dr. N. F. Childers of the Federal Experiment Station.

also been suggested that flower formation in the pineapple may be a response to relatively low temperature (**6**).

Which *internal* factors make the pineapple plant flower during the normal season? Since as little as 0.05 mg. per plant of naphthaleneacetic acid (fig. 2, the 1 p.p.m. curve) is in some cases sufficient to force a plant into flowering, it is tempting to attribute the normal flower production to increased hormone activity. Auxin analyses of the pineapple plant are necessary to provide further evidence. Such an analysis is feasible at present, because a convenient extraction method exists (**12**) for the determination of free auxin in plant materials.

It should not be overlooked, however, that the level of nutritional materials, such as carbohydrates and nitrogenous compounds, may undergo critical changes during the winter season which bring about flower induction. NIGHTINGALE's detailed observations of the Smooth Cayenne in Hawaii (**6**) have shown that plants deficient in nitrate and high in carbohydrates may fruit months earlier than others that contain more liberal nitrate reserves but a lower concentration of carbohydrates. It is not unlikely that a co-ordinated action of hormones of the auxin type and of nutritional factors determines the flowering of the pineapple plant, just as such action determines root formation in cuttings (**11**, **13**).

PRACTICAL ASPECTS

By making use of diagrams such as presented in figures 6–8, the pineapple-grower can, with the aid of flower-inducing chemicals, control not only the time of harvest but the weight of his fruits as well. Since the vegetative growing-point changes into a floral apex after treatment, no additional foliage leaves are formed. Thus, for all practical purposes, the plant has to nourish its fruit with the leaves it has at the time of treatment.

When, for the particular conditions of a plantation, the leaf-number/fruit-weight diagram has been worked out, the weight of the fruit at harvest time can be predicted at the time of treatment. In addition, when a certain fruit size is desired, this can be obtained by treating the plants at a stage when they have a number of leaves which in the diagram corresponds to the desired fruit size. For example, when plants are growing under the conditions of figures 7 and 8, and a fruit weight of 3 kg. is desired, plants having between twenty-one and thirty-one leaves should be treated. When a smaller fruit size is desired, for instance, 2 kg., the plants should be treated when they have between sixteen and twenty-six leaves.

The experiments reported here with Cabezona pineapples demonstrate how controlled pineapple production throughout the year is possible. In these experiments, as demonstrated above, fruits weighed no less when produced out of season.

In order to have fruits of the desired size throughout the year, it is essential to have plants available of a size suitable for treatment throughout the year. This is possible for the Cabezona, which flowers with difficulty during the natural flowering season, because no undesirable flowering of immature plants takes place. A plant which flowers prematurely is lost for production.

In the opinion of the writer the low fruit weights which are reported when the Red Spanish variety is forced into flower outside the natural season are due to the fact that the plants treated are not sufficiently mature to support a large fruit. A continuous supply of plants

suitable for treatment is not available in this readily flowering variety because of loss due to the flowering of immature plants during the winter season.

For production throughout the year, without an accompanying fruit loss during the out-of-season months, it is therefore necessary to prevent undesired flowering of immature plants. This may be accomplished by using varieties such as the Cabezona which have a natural tendency to limited flowering, or by preventing flowering in the easy-flowering varieties by physiological means.

Summary

1. In the Cabezona variety of pineapple naphthaleneacetic acid (NA) can induce flowers in every month of the year (fig. 1).

2. Amounts of NA as small as 0.05 mg. per plant are capable of inducing flowers in a limited number of plants (1 p.p.m. curve of fig. 1), while 0.25 and 0.5 mg. per plant cause practically 100% flowering.

3. Fruit weight depends upon the size of the plant. The correlation between the number of leaves of a plant and the weight of the fruit it produces was determined (figs. 6–8).

4. Although 2,4-dichlorophenoxyacetic acid (2,4-D) has flower-inducing powers equal to NA (fig. 5), fruit induced by 2,4-D shows a lower average fruit weight as compared with fruit induced by NA (fig. 8 and table 2). This may be due to the adverse effect of 2,4-D on the development of young leaves.

5. Preliminary data indicate that the pineapple may be a short-day plant and that long-day conditions prevent natural flowering but not flowering induced by NA.

6. A method is outlined which makes fruit production in pineapples possible throughout the year without the loss of fruit weight now experienced by commercial growers when they force plants into flower out of season.

INSTITUTE OF TROPICAL AGRICULTURE
MAYAGÜEZ, PUERTO RICO

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A COMPARISON OF HISTOLOGICAL RESPONSES OF BEAN PLANTS TO TRYPTOPHANE AND TO LOW CONCENTRATIONS OF INDOLEACETIC ACID

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 574

MARY AILEEN MURRAY AND A. GERALDINE WHITING

Introduction

Tryptophane is one of the many chemical compounds which have been studied as growth-regulating substances. Its effects, however, have been questioned. WENT and THIMANN (9) considered tryptophane itself an inactive compound which is changed into an active substance in the plant. They stated that if tryptophane is applied to *Avena*, curvatures result in approximately 2 hours. During this period, they presumed, the tryptophane is converted into indoleacetic acid. The basis for this assumption is the known conversion of tryptophane into indoleacetic acid by bacteria and fungi. More recent chemical evidence on the problem has been presented by AVERY and BERGER (1), certain of whose experiments showed a significant discrepancy between the low yield (0.015%) of indoleacetic acid from pure tryptophane and the higher yield (0.022%) of indoleacetic acid from the natural precursor in plant tissues (crude corn). From these experiments and from other reports, AVERY and BERGER felt safe in concluding that tryptophane is not identical with the corn auxin precursor to which most of the activity is attributable but that tryptophane is a plant auxin precursor of a low degree of activity.

Both indoleacetic acid (5, 7) and tryptophane (6) have been studied in a series of experiments testing the growth-regulating effects of various compounds applied in lanolin to the cut surface of the decapitated second internode of young kidney-bean plants. KRAUS (6) stated that tissue responses of the bean stem to application of tryptophane are similar in some respects to response to indoleacetic acid but are very different in many other respects. These differences seem to invalidate the assumption that tryptophane acts as a growth-regulating substance only after conversion to indoleacetic acid. He compared responses obtained from applications of 3 and 1.5% indoleacetic acid with the reactions resulting from treatment with 2% tryptophane. Since recent studies (1) have shown that the conversion of tryptophane results in a relatively small percentage of indoleacetic acid, the concentrations of indoleacetic acid used by KRAUS are far above those which might be expected from conversion of 2% tryptophane. Consequently, the question arises: Would the response induced by 2% tryptophane resemble more closely that induced by a lower concentration of indoleacetic acid?

The purposes of this paper are (*a*) to report the results of histological studies with five concentrations of indoleacetic acid—0.2, 0.02, 0.002, 0.0002, and 0.00002%—and (*b*) to compare these histological responses with those described by KRAUS for tryptophane. The materials and the methods are the same as those used in earlier studies of indoleacetic acid (7) and of tryptophane (6).

Results

Following decapitation and treatment with indoleacetic acid, proliferation of many of the cells at the cut surface takes place. This applies especially to those cells which are parenchymatous or incompletely differentiated. As general proliferation progresses, the normal pattern of tissue organization is quickly lost. It is this growth which forms the tumor above the cut surface. Below the zone of general proliferation the pattern of responding tissues is similar to the normal tissue arrangement in the stem. It is perhaps in this region that the most characteristic features of the histological effects of the various growth-regulating substances are to be found, although the character of the tumor may likewise be distinctive. With increasing distances from the surface of application, the response becomes less pronounced, until a level is reached beyond which no effect is evident. The degree of reaction varies among individual plants and from bundle to bundle within the stem, major bundles showing the greatest degree of reaction. Environmental factors also influence the response.

From gross observations and from preliminary studies of histological material for the five concentrations of indoleacetic acid, it is evident that response is greatest with 0.2%, less with 0.02%, and only slight with 0.002%. The two lowest concentrations (0.0002 and 0.00002%) are apparently below the threshold of effectiveness for indoleacetic acid to induce histological changes or tumor formation under the conditions of this experiment (fig. 13*C*). Response to these two concentrations is the same as that which may result from decapitation and application of pure lanolin. The essential features of the histological responses to the three effective concentrations are summarized in table 1. Supplementary details and variations are reported in the following paragraphs.

At the cut surface the epidermal cells are injured (fig. 9), and those cells immediately adjacent may enlarge slightly and divide in radial, transverse, or tangential planes. This response is limited in extent and may occur in any one of the concentrations of indoleacetic acid or in control material. Below this level the epidermis shows the usual adjustment to increase in stem diameter. However, with the extreme enlargement at points of root formation, rupture of the epidermis may occur.

Response in the cortical parenchyma is correlated with the degree of concentration. With 0.2% indoleacetic acid the outer cortical parenchyma cells become conspicuously enlarged at the upper levels (fig. 7*A*). Cell division is infrequent. The inner parenchymatous cells, especially those directly adjoining the endodermis, become meristematic (figs. 2*A*, 3*A*) and, like it, form part of the capping tissues over root primordia (figs. 3*B*, 7*A*). Otherwise the derivatives mature as parenchyma. Cortical response is most pronounced in the upper 2 mm. of the stem tip and progressively decreases to the 3- or 4-mm. level. With the 0.02% concentration, cell enlargement is distinctly less (fig. 4*A*). There may be a few divisions in the inner cells, but they do not become highly meristematic. This response extends approximately 1 mm. from the cut surface, with only traces below this. By the 4th day derivatives are maturing as parenchyma (fig. 5*B*). In the lowest effective concentration (0.002%) there appears to be no response in the cortical parenchyma (fig. 4*B*).

The endodermis is the most highly re-

sponsive tissue. By the end of 24 hours meristematic activity is induced by all three concentrations which cause any response. With the highest concentration (0.2%) the endodermis shows an increasing number of tangential divisions by the 2d day (figs. 1*B*, 3*A*). By the 3d day it has become a relatively wide band of meristematic tissue (fig. 2*A*) with the initiation of root primordia under way at points of intense activity (fig. 3*B*). The zone of root formation and intense reaction extends to approximately 1 mm. below the cut surface. Lower than this the degree of meristematic activity decreases rapidly. Tangential divisions are fewer and the characteristic radial arrangement is more evident. Traces of response are found 5–6 mm. below the treated surface. Although the upper levels may remain meristematic in areas associated with root formation or vascularization (fig. 5*A*), evidence of maturation of the derivatives into parenchyma generally occurs by the third or fourth day. The formation of small vascular strands is initiated during the period of meristematic activity, and divisions in various planes result in patches of small cells. By the 3d or 4th day certain of the cells in the center of the patch may mature as reticulate tracheids, whereas peripheral cells remain meristematic (figs. 6*A*, 12*B*). The size and organization of these vascular strands increase until, in some stems, large bundles are formed (fig. 17*D*). Endodermal bundles are characteristic of the upper millimeter of the stem tip. Below this a few strands may end blindly in the endodermis, but others pass from the endodermis across the pericycle into the phloem.

Endodermal response at uppermost levels to the 0.02% concentration is similar to that below the zone of root formation with the highest concentration. Response is initiated by the end of 24 hours and consists mainly of tangential divisions which form a band of active tissue. At upper levels this band may be as much as eight cells deep over the major bundles of the stem (figs. 1*C*, 2*B*). At the depth of 1 mm. the number of endodermal derivatives decreases, and only traces of activity are found below 2 mm. Root primordia were not observed in any material treated with this concentration. By the 3d or 4th day derivatives begin to mature. This is made evident by the rounding-up of cells as parenchyma or by the occasional formation of reticulate tracheids and weakly organized vascular strands (figs. 5*B*, 7*B*, 13*A*). Vascularization follows the same course as in stems treated with the higher concentrations. The next lower concentration—0.002% —induces noticeable response only in the endodermis at uppermost levels. By the end of 24 hours tangential endodermal divisions are evident over two or three major bundles of the stem, frequently only along the sides of these bundles. By the 2d day the number of divisions may increase slightly (fig. 1*D*), after which there is little change (figs. 5*C*, 13*B*). A depth of 1 mm. seems to be the level of histological response farthest from the surface of application (figs. 4*B*, 6*C*). The derivatives mature as parenchyma (fig. 2*C*).

The pericycle responds in a limited manner. At the upper levels with the two higher concentrations, the cells enlarge, often proliferate slightly, and mature as parenchyma (figs. 3*A*, 4*A*, 7*A*, 9). At lower levels the pericyclic cells mature as the usual thick-walled fibers. The development as fibers is fairly well established at 3 mm. with the 0.2% concentration. With the two lower concentrations, well-formed fibers may be observed at less than the 1-mm. level (fig. 6*B*, *C*).

Crushed pericyclic cells were only rarely observed.

The primary phloem is nearly as sensitive as the endodermis, although the reaction is never as conspicuous. The initial stages of response are cell enlargement and an increasingly meristematic condition, which at the cut surface results in general proliferation (figs. 4*A*, *B*, 9). The greatest activity is found with the 0.2% concentration. The primary phloem parenchyma becomes highly meristematic and generally indistinguishable from active endodermal and ray tissues at those levels in which root formation takes place (fig. 2*A*). Below the root zone (somewhat below 1 mm.) the organization of tissue areas is re-established and a lesser degree of proliferation in the phloem is evident. By the 4th day the derivatives, except those in the root zone, are maturing as parenchyma or are differentiating as vascular elements. With the 0.02% concentration the effects in the phloem are similar to those with the higher concentration at levels below the zone of root formation. Enlargement and cellular proliferation were observed to levels 2 mm. deep. The extent of the histological response to the 0.002% concentration is general proliferation at the cut surface.

Vascularization of the primary phloem parenchyma commences with the organization of groups of small meristematic cells, similar to those found in the endodermis. With the 0.2% concentration some of these cells may differentiate as short reticulate tracheids, and the organization of a vascular strand may or may not follow. Poorly organized vascular strands were observed at the 0.5-mm. level (fig. 5*A*). They are better organized at lower levels (figs. 12*A*, 14*A*). As has already been noted, these vascular bundles may be in the endodermis at upper levels, with their course converging inward to pass across the pericycle, possibly at the 1-mm. level, where these cells are meristematic, and thence into the phloem (fig. 9). Within the phloem these vascular strands extend downward for 1 mm. or more. Many of these bundles terminate in direct inward connections through a phloem ray with the secondary phloem, cambium, and secondary xylem of the stele (figs. 10*A*, *B*, 11); other bundles may end blindly in the phloem. Branching of these vascular bundles may occur, especially toward the upper levels, where they pass outward through the pericycle into the endodermis. The origin, structure, and course of vascularization with the 0.02% concentration are similar.

The secondary phloem is relatively unaffected by the various concentrations. At levels just below the cut surface, the parenchyma and immature elements may proliferate (fig. 4*A*). Response below this general proliferation is limited to parenchymatous elements which may show a slight degree of cell enlargement (fig. 2*B*).

The cambium, like other undifferentiated tissues, tends to proliferate at the cut surface. Below the level of application the degree of activity is greatest with the 0.2% concentration and less with 0.02%; with 0.002% it is no greater than that found in stems decapitated and treated with pure lanolin. The zone of undifferentiated cells about the cambium becomes proportionately wider. Maturation of these undifferentiated cells indicates that the larger number of derivatives is cut off toward the xylem.

Xylem vessels and tracheids lignified previous to treatment remain relatively unchanged. However, xylem parenchyma and incompletely mature elements (fig. 4*A*) at the cut surface proliferate.

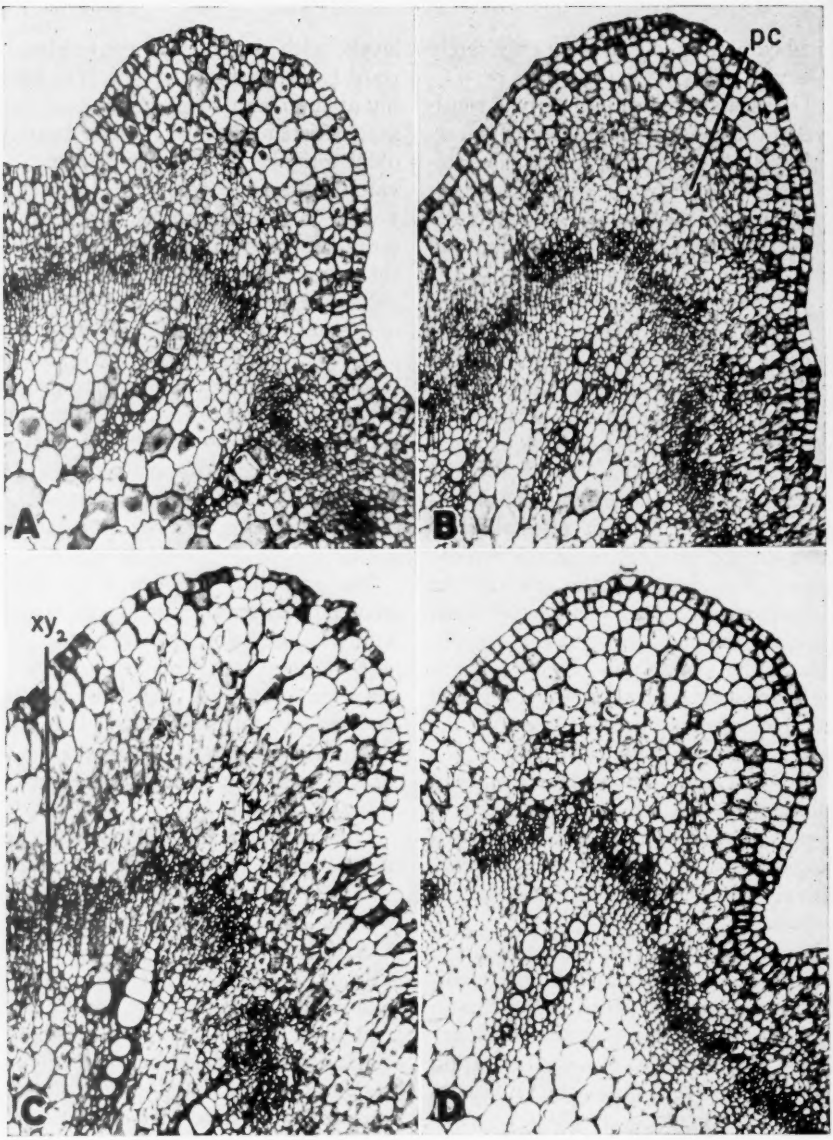

FIG. 1.—Transections of decapitated stems, 0.5 mm. below cut surface: *pc*, pericycle; xy_2, secondary xylem. *A*, 1 day after treatment with 0.2% indoleacetic acid. Cells of inner cortical parenchyma and endodermis dividing; primary phloem parenchyma meristematic; increased activity in cambium. *B*, 2 days, 0.2%. Endodermis forming meristematic band; pericycle and primary phloem parenchyma cells enlarged and meristematic; secondary xylem tracheids differentiating. *C*, 2 days, 0.02%. Similar to *B* but less in extent. *D*, 2 days, 0.002%. Some tangential divisions in endodermis; otherwise little response.

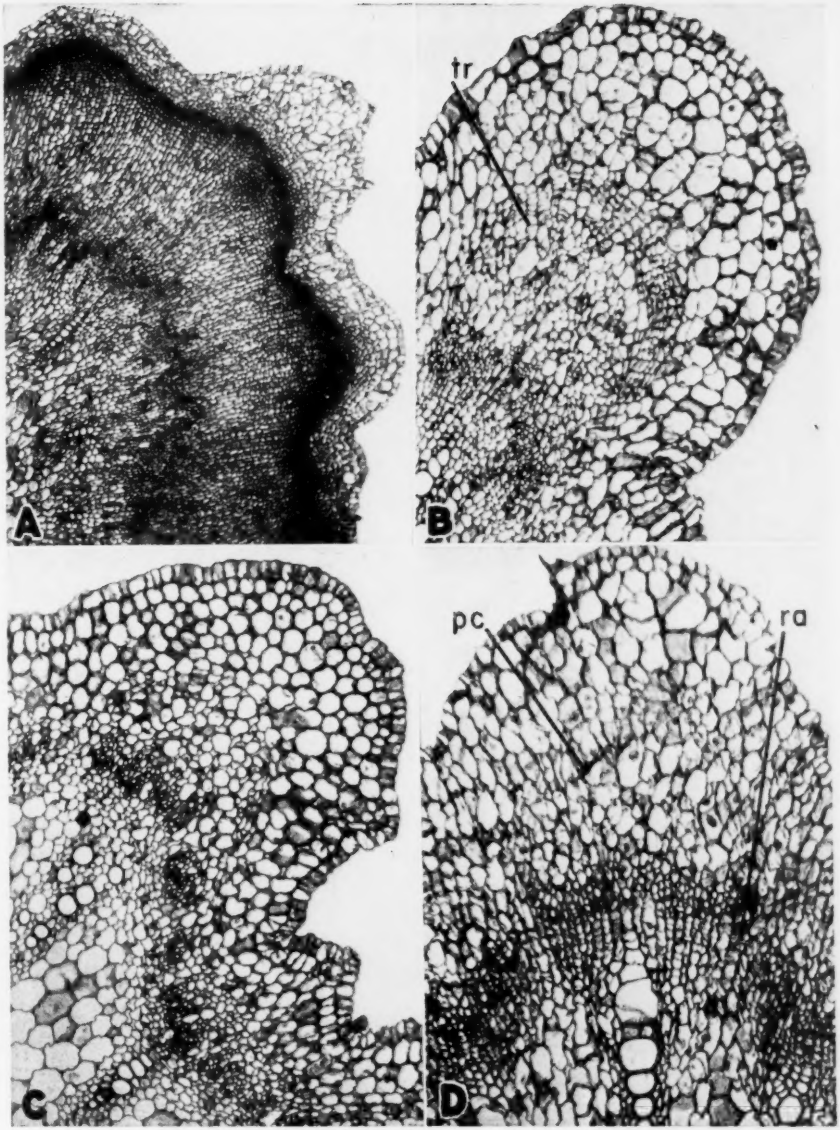

FIG. 2.—Transections of stems, 3 days after treatment, 0.5 mm. below cut surface: *tr*, tracheid; *ra*, ray. *A*, 0.2% indoleacetic acid. Highly meristematic band of proliferated endodermis, inner cortical parenchyma, pericycle, and primary phloem and ray parenchyma. Proliferation of pith parenchyma commencing centrad to protoxylem points. *B*, 0.02%. Maturation commencing with differentiation of some tracheids; other cells maturing as parenchyma. *C*, 0.002%. Response no greater than at 2 days. *D*, 2% tryptophane. Early stage in proliferation of endodermis; pericycle crushed (*pc*); little response in phloem; interfascicular cambium developing in ray (*ra*).

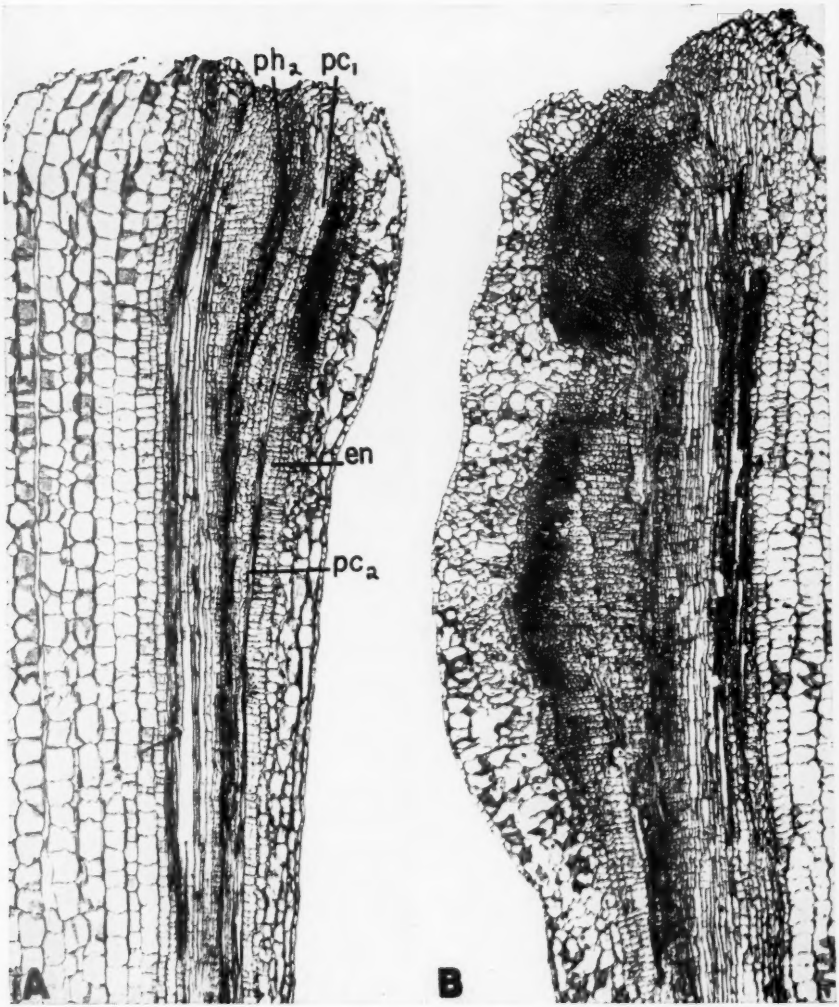

FIG. 3.—Longisections, 0.2% indoleacetic acid: *en*, endodermis; ph_2, secondary phloem. *A*, 2 days. Cells of outer cortical parenchyma greatly enlarged, inner ones proliferating; at upper levels in endodermis initiation of root primordium, at lower levels cell elongation and tangential divisions; at upper levels pericyclic cells (pc_1) enlarged and proliferating, at lower levels pericyclic fibers (pc_2); marked activity in primary phloem and cambium at upper levels. *B*, 3 days. Response similar but greater in degree than at 2 days; several root primordia evident; cambial derivatives differentiated as secondary xylem tracheids.

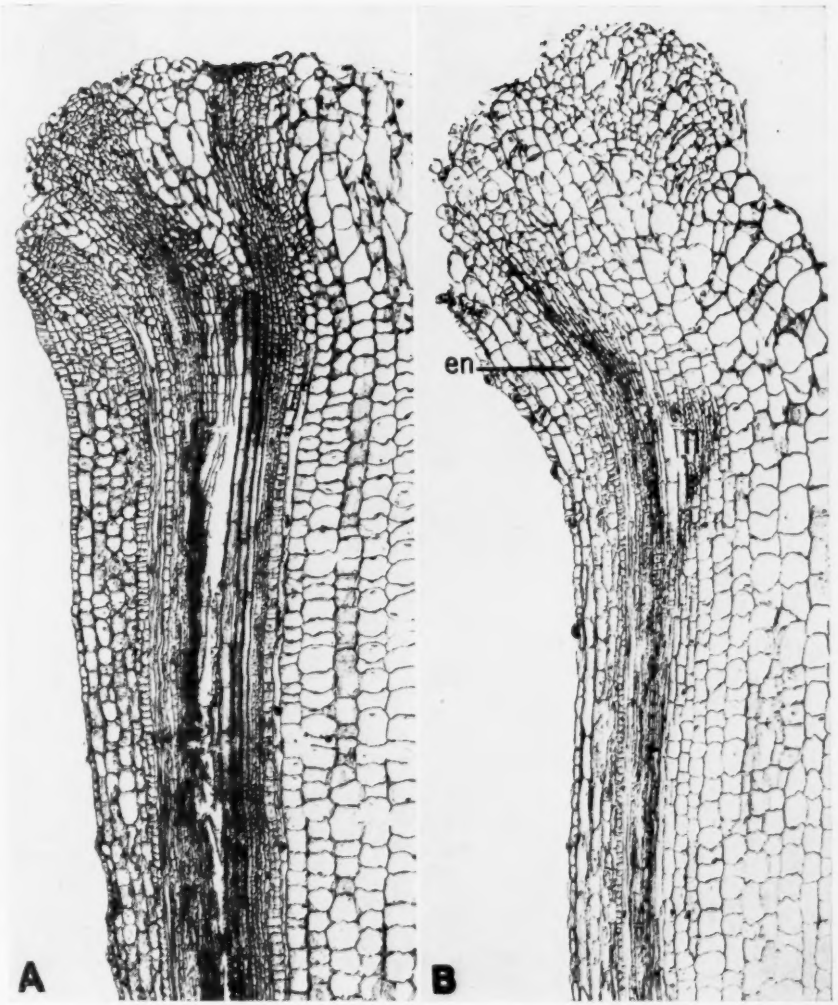

FIG. 4.—Longisections, 3 days after treatment. *A*, 0.02% indoleacetic acid. Across cut surface general proliferation of tissues except epidermis; conspicuously large cells are proliferated immature tracheal elements; below cut surface slight proliferation in cortical parenchyma, endodermis, pericycle, and primary phloem parenchyma. *B*, 0.002%. Similar proliferation of tissues at surface of application. Section through side of bundle showing slight proliferation in endodermis. (Compare with lateral position of proliferated endodermal cells in fig. 1*D*.)

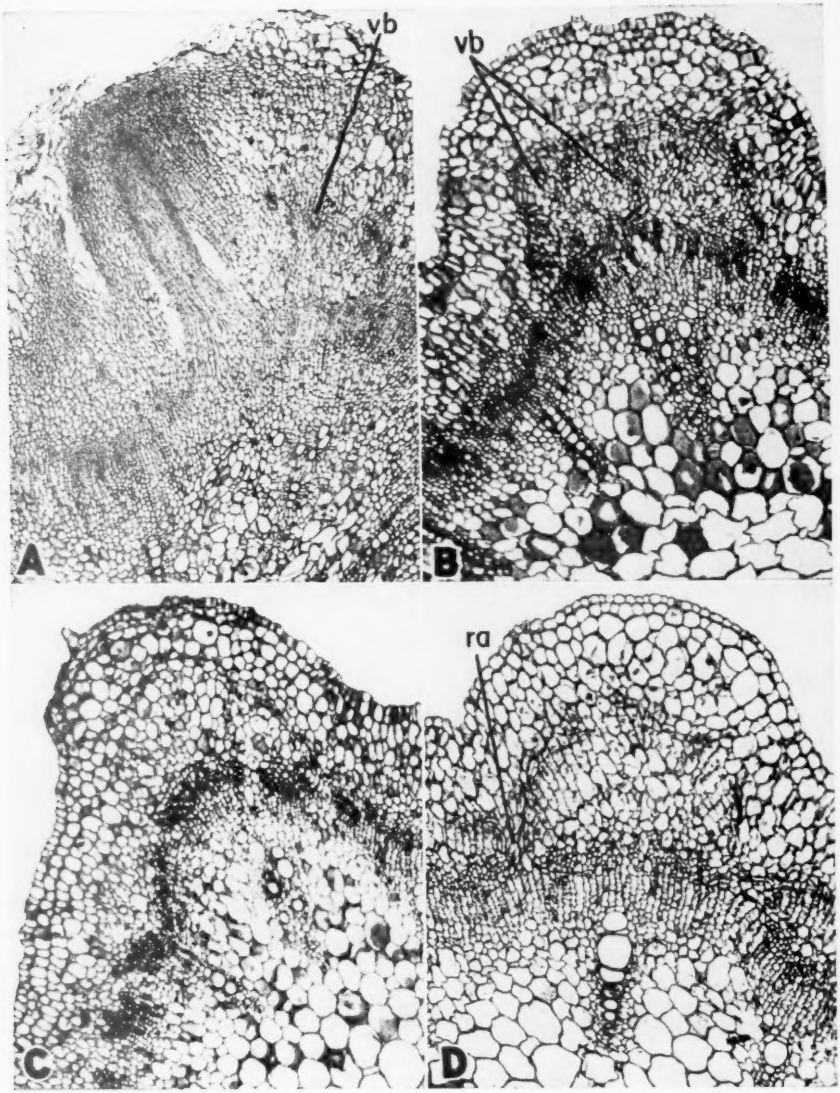

FIG. 5.—Transections 0.5 mm. below cut surface, 4 days after treatment: *vb*, vascular bundle. *A*, 0.2% indoleacetic acid. Root primordium and associated tissues meristematic, other tissues maturing; formation of vascular strands (*vb*) in primary phloem parenchyma commencing; numerous scattered secondary tracheids differentiated in xylem; proliferated ray and pith parenchyma differentiating tracheids and vascular strands. *B*, 0.02%. Similar to 3 days, more mature; vascularization (*vb*) of endodermis and phloem, strands poorly organized. *C*, 0.002%. Similar to 2-day response. *D*, 2% tryptophane. Continued proliferation in cortical parenchyma and endodermis; primary phloem parenchyma slightly meristematic. Note initiation of cambial strands in endodermis along sides of bundles and in meristematic ray (*ra*).

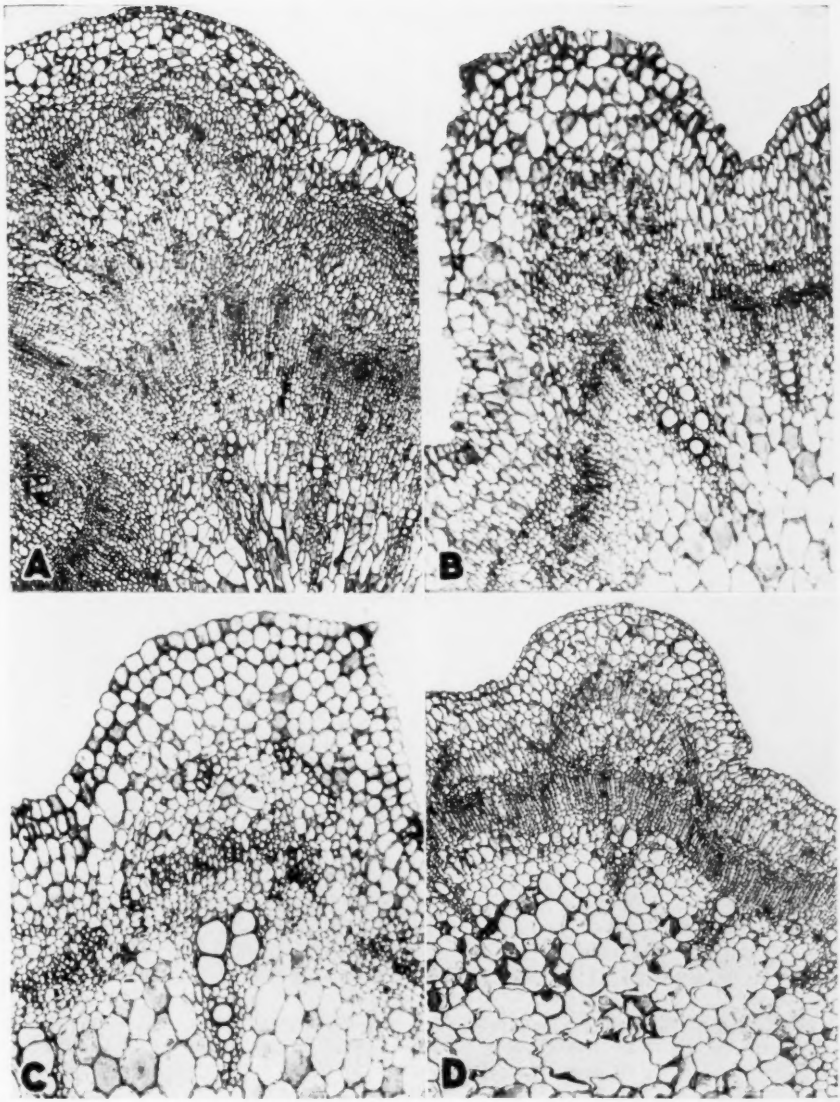

FIG. 6.—Transections 1 mm. below cut surface, 4 days after treatment. *A*, 0.2% indoleacetic acid; *B*, 0.02%; *C*, 0.002%; same stems respectively as in fig. 5. *D*, 2% tryptophane. Degree of response with each treatment somewhat less at this lower level.

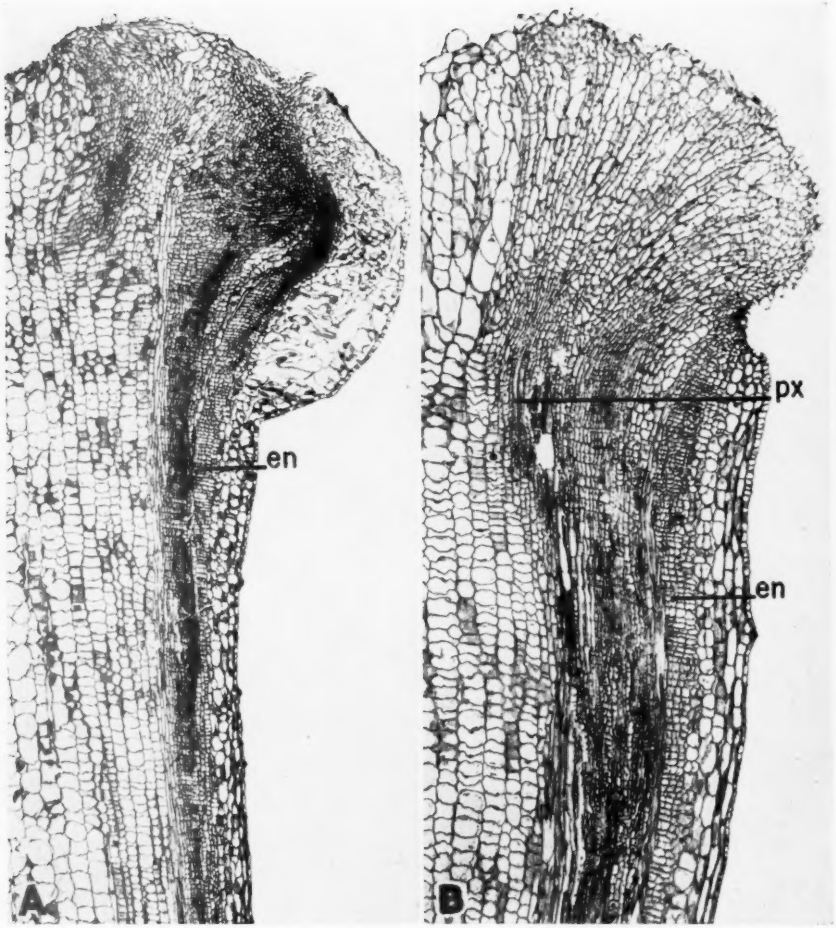

FIG. 7.—Longisections, 4 days after treatment: *px*, protoxylem. *A*, 0.2% indoleacetic acid. Section in ray showing relationship of tissues in development of root primordium; proliferation of marginal pith at upper level. *B*, 0.02%, early stages in development of tumor above cut surface. Note active proliferation of central pith; proliferated tissues below cut surface generally maturing.

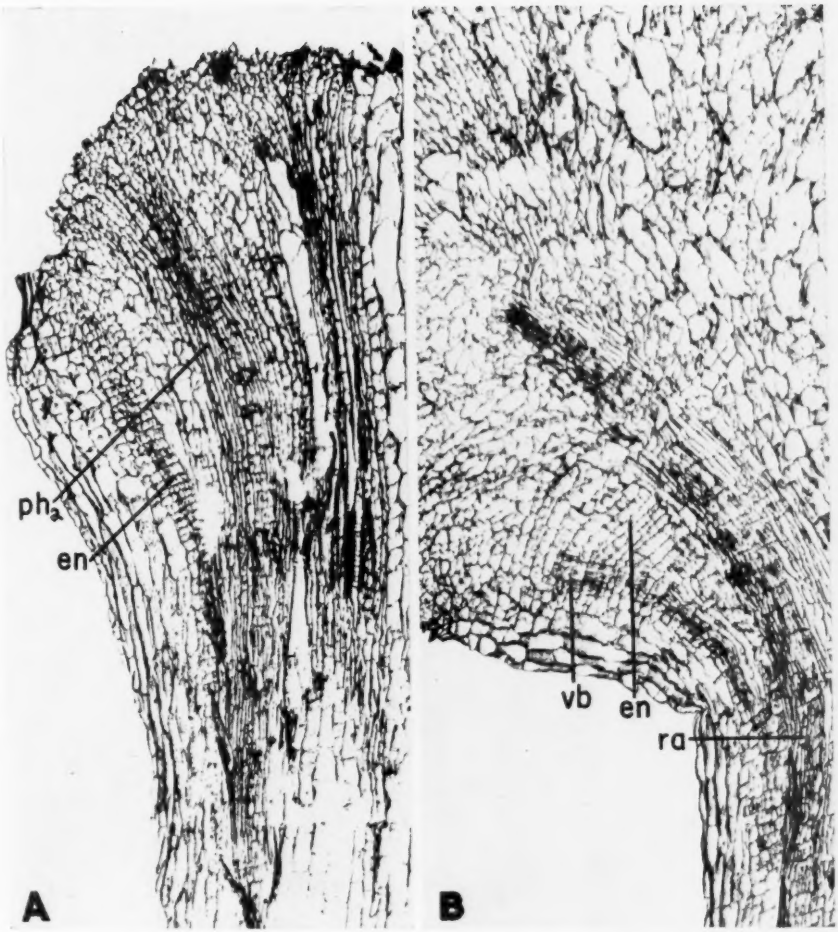

FIG. 8.—Longisections of stems treated with 2% tryptophane. *A*, 4 days after treatment. Early stage of tumor; active proliferation of tissues at lower levels; pith disintegrating. Compare with more advanced response in fig. 7*B*. *B*, 7 days. More advanced stage in formation of tumor; greatly proliferated endodermis, derivatives differentiated as vascular strand (*vb*), elements of which connect with stele through derivatives of ray (*ra*).

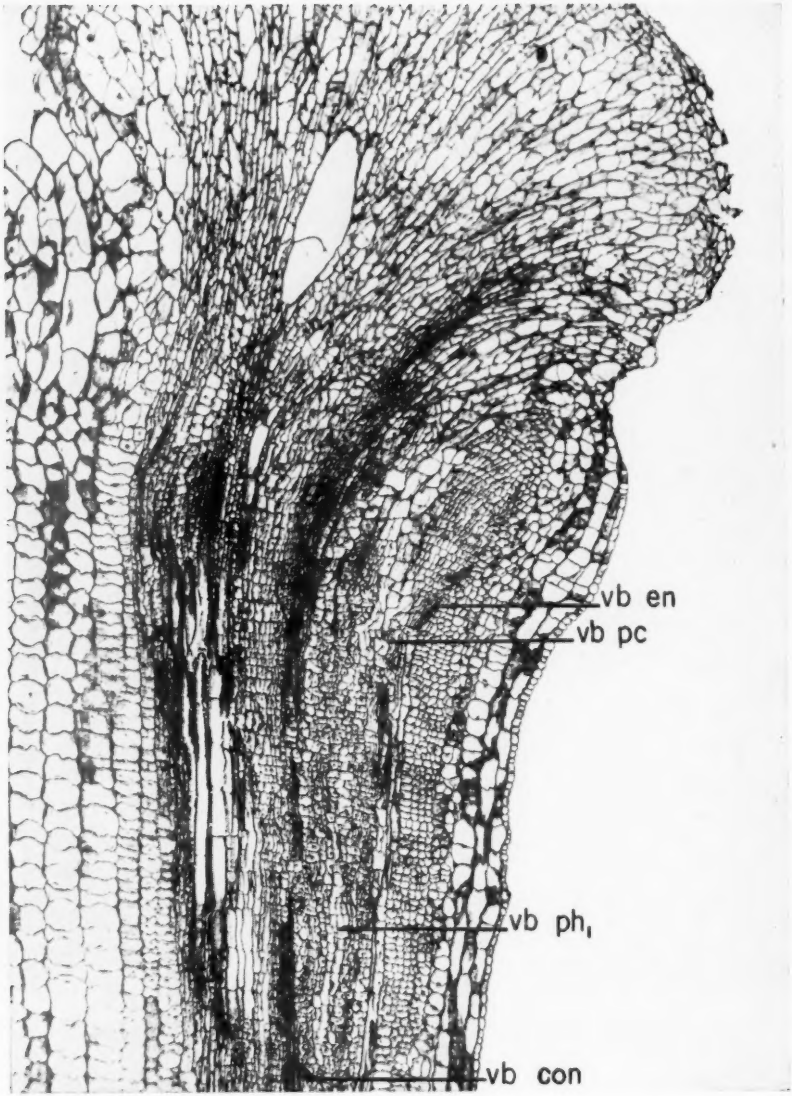

FIG. 9.—Longisection 6 days after treatment with 0.2% indoleacetic acid, showing course of vascularization: *vb con*, vascular connection; ph_1, primary phloem. At upper levels vascular bundle differentiated in derivatives of endodermis (*vb en*); at 1 mm. in proliferated pericyclic cells (*vb pc*); at lower levels in derivatives of primary phloem parenchyma (*vb* ph_1); at 2.5 mm. vascular bundle connects directly inward (*vb con*). See fig. 10*A*.

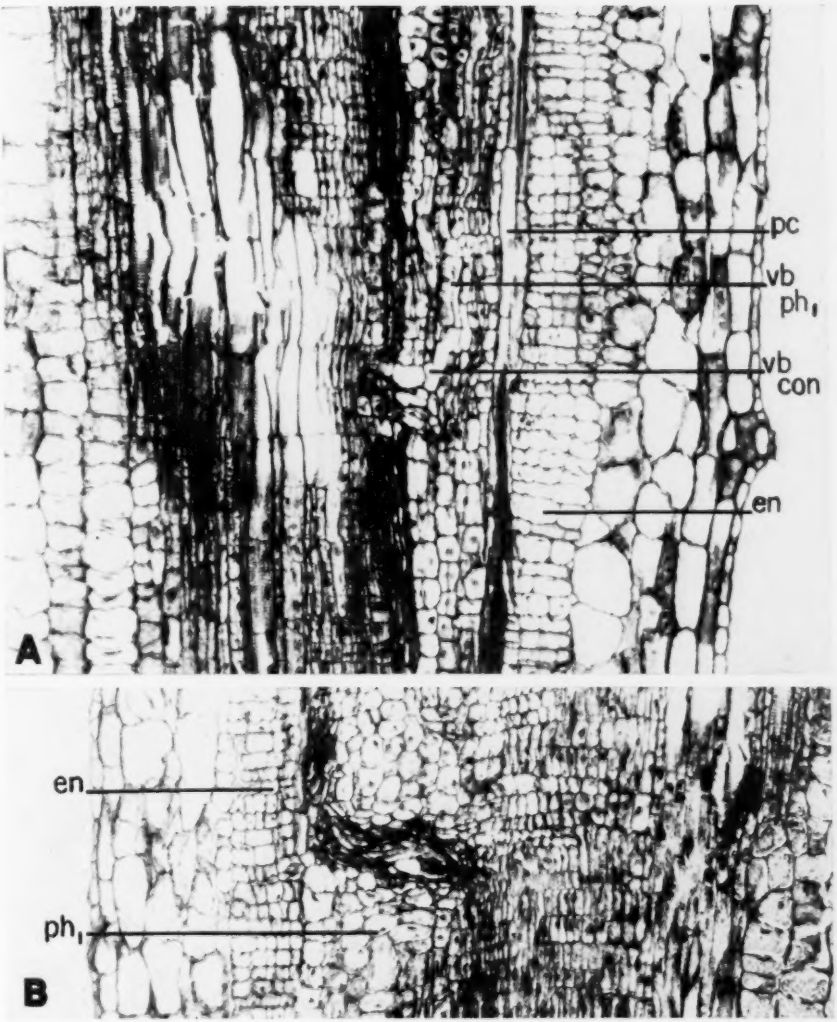

FIG. 10.—Longisections, 0.2% indoleacetic acid. *A*, 6 days after treatment, same stem as fig. 9. Cellular detail of connection (*vb con*) between vascular tissues of primary phloem bundle (*vb* ph_1) and secondary phloem, cambium, and secondary xylem of stele. *B*, 18 days after treatment. Detail of similar connection in another stem.

FIG. 11.—Transection 9 days after treatment with 0.2% indoleacetic acid, 2.5 mm. below cut surface. Same type of vascular connection as in fig. 10*A*. Endodermal derivatives matured as parenchyma; scattered tracheids in wide band of secondary xylem; medullary and xylem rays conspicuous.

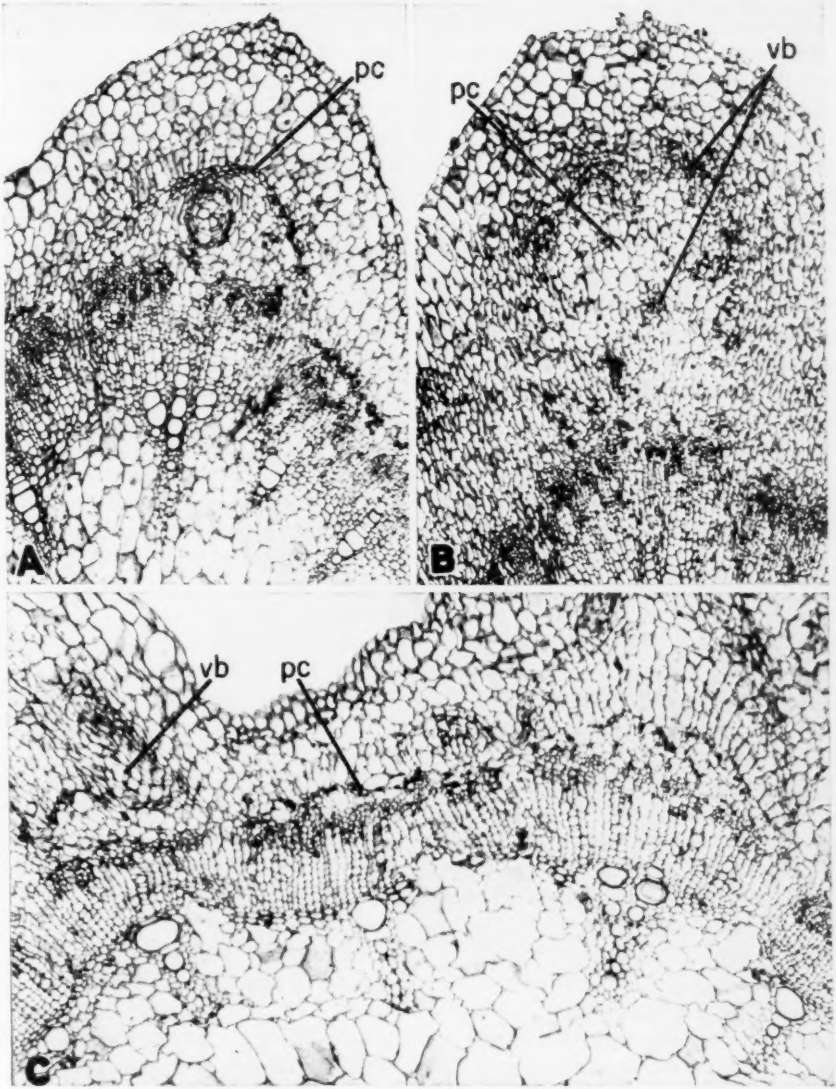

FIG. 12.—Transections, 9 days after treatment. *A*, 0.2% indoleacetic acid, 2 mm. below cut surface, same sector of stem as fig. 11. Clearly organized bundle in primary phloem parenchyma. *B*, same stem as *A*, 1 mm. below cut surface. Branches (*vb*) of same bundle, at this level in endodermis and primary phloem. *C*, 2% tryptophane, 1 mm. below cut surface. Vascularization (*vb*) in proliferated endodermis along flanks of bundles; no proliferation or vascularization in phloem or pith.

The greatest response is found in the differentiation of the cambial derivatives formed after treatment. At the highest concentration these derivatives form a proportionately wide band of short, reticulately thickened tracheids intermingled with occasional vessels and numerous ray cells (fig. 11). At 0.02% this band is similar but less wide (fig. 1*C*), and at 0.002% only traces of a tracheid layer are evident. The total downward extent of this response is difficult to determine as it blends with the secondary growth of unaffected portions of the stem.

On the basis of behavior a ray may be divided into an outer portion flanking the phloem and a portion flanking the xylem. With the 0.2% concentration the outer portion becomes an active part of the band of meristematic tissues in the upper 2 mm. of the stem tip (fig. 2*A*). The importance of the ray in the formation of root primordia has already been described in detail (**4**). Below the level of root formation the outer portion of the ray is very much less active, and its derivatives mature as parenchyma. An interfascicular cambium may or may not be formed. The response to 0.02% indoleacetic acid is similar to that below the root zone for the higher concentration. The lowest effective concentration (0.002%) induces no marked histological change in the ray.

The inner portion of the ray is associated with the pith, and the two may be described together. With both the 0.2% and the 0.02% concentrations (figs. 2*A*, 6*A*), response is delayed and proliferation is not initiated until the 3d or 4th day. Both tissues remain inactive with the 0.002% concentration. Large parenchymatous cells of the inner portion of the ray and of the marginal pith (pith abutting the rays or that centrad to the protoxylem points) may divide several times. The derivatives mature as parenchyma or as reticulate tracheids, or they remain meristematic to form vascular strands. The intensity of this reaction is greater at the 0.2% concentration, but with both concentrations the response is confined to the uppermost levels. It reaches slightly deeper than 1 mm. below the cut surface with 0.2% indoleacetic acid (fig. 15*A*) and less than 1 mm. for the other concentration. The central pith persists as a functional tissue. At the cut surface it proliferates but retains its parenchymatous character (fig. 15*B*).

The upward thrust of proliferating tissues to form the tumor above the cut surface commences by the 3d day (figs. 3*B*, 4*A*, *B*). Tissues from the pericycle outward participate but little in the growth of the tumor (figs. 7*B*, 15*B*). Proliferation above the vascular elements is greatest during the early stages, and derivatives form the peripheral portion of the tumor (figs. 9, 15*B*). In mature stages of the relatively small, dome-shaped tumor formed in response to 0.02% indoleacetic acid, proliferated pith forms the central portion (fig. 15*B*). With 0.2% the very large, cone-shaped tumor is formed almost entirely of proliferated pith (fig. 15*A*). The heavy marginal band of vascular tissue (fig. 17*A*) is connected with both the endodermal vascular system and the central stelar structure (fig. 17*A*, *B*, *C*, *D*). The anastomosing vascular strands in the center extend downward and connect directly with vascular strands arising in the pith (fig. 15*A*). With the lower concentration (0.02%), there is little vascularization and the tumor is mainly parenchymatous in composition. With the other three concentrations (0.002, 0.0002, and 0.00002%), the form and structure of the growth above the cut surface is similar to

those tumors which may be formed after decapitation and the application of pure lanolin (fig. 16*A*, *B*).

Discussion

A comparison of responses reported in this paper with those described earlier by KRAUS, BROWN, and HAMNER (7) clearly indicates that the four highest concentrations of indoleacetic acid (3.0, 0.2, 0.02, and 0.002%) induce responses which are similar in character and differ essentially only in degree. Successive decrements in concentration result in a series of decreasing expressions of response. Likewise, decapitated stems treated with higher concentrations of indoleacetic acid show a series of decreasing reactions at successive levels below the treated surface. Comparison of these two series shows that responses induced at successively lower levels in a stem tip treated with a high concentration are similar to responses at higher levels induced by treatment with the lower concentrations. The similarity between these two series suggests dependence of expression of response upon a concentration gradient of indoleacetic acid. On this basis, the absence of root primordia following application of lower concentrations should be expected rather than considered a striking dissimilarity. Apparently a certain intensity or threshold of reaction must be reached before development of adventitious roots occurs. With a concentration of 3% indoleacetic acid, this intensity is maintained a distance of several millimeters below the cut surface, and numerous root primordia are formed. When a stem tip is treated with 0.2% indoleacetic acid, fewer root primordia are developed, they are found within approximately the first millimeter below the cut surface, and the reaction within this limit is comparable with that at the base of the root zone with 3% indoleacetic acid. No root primordia are formed with the application of 0.02% indoleacetic acid. The maximum response is comparable with levels immediately below the root zone with applications of 0.2% indoleacetic acid. A concentration of 0.002% indoleacetic acid induces mere traces of response even at the surface of application. In appearance these responses are similar to the responses at the lowest levels with higher concentrations. The response of decapitated bean plants to treatment with various concentrations of indoleacetic acid follows a consistent pattern and varies only in degree.

BEAL (4) has observed that, for the occurrence of a response, a certain concentration gradient of the indoleacetic acid must be established downward from the treated surface. He found, however, that the nutritional status of the tissues was closely associated with this gradient. Treated segments placed on nutrient agar lacking either sugar or nitrogen showed no gross enlargement or proliferation. Other writers (2, 3, 8) have also demonstrated very close relationship between the carbohydrate content of the tissues and the effectiveness of a given growth substance. On this basis, it is conceivable that a plant with limited supplies of carbohydrates and treated with a high concentration of indoleacetic acid could simulate the histological response of a plant containing carbohydrates in greater amount but treated with a low concentration of indoleacetic acid. It is evident that a comparison of concentrations and degrees of response can be made only by the use of plants which are uniform, or nearly so, in their growth status and in their chemical composition under identical or closely similar environments.

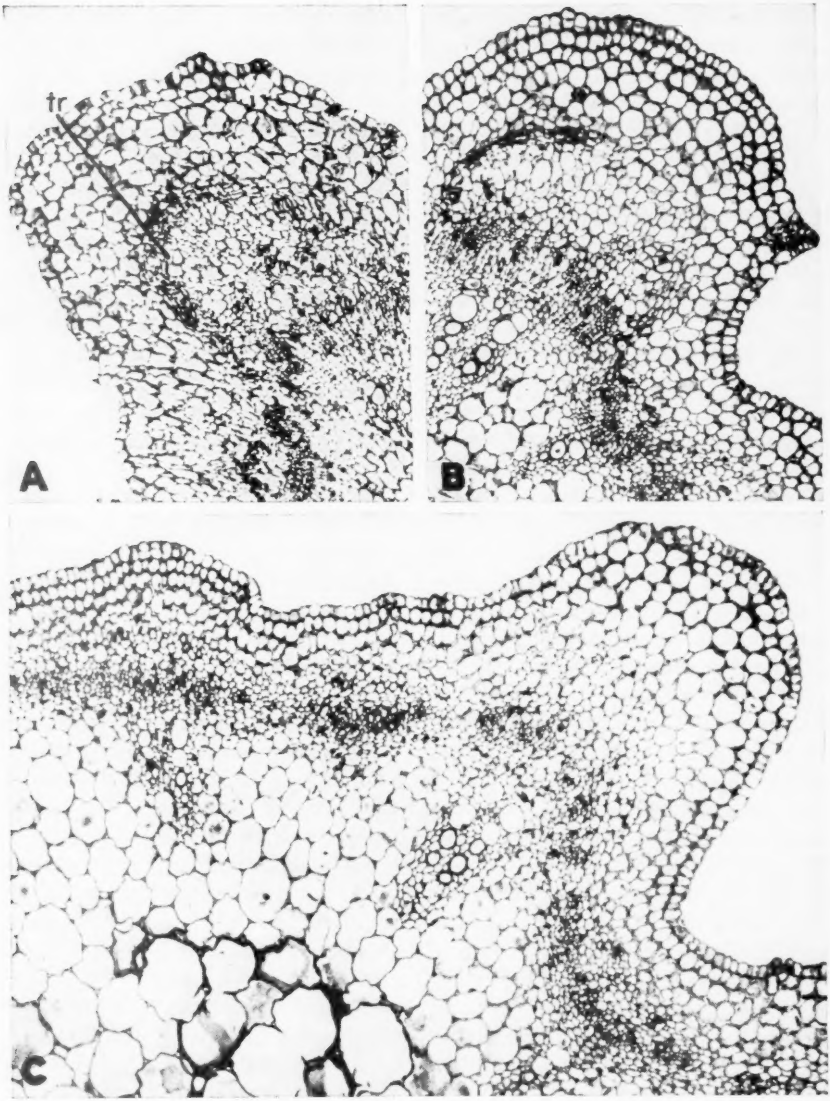

FIG. 13.—Transections 9 days after treatment. *A*, 0.02% indoleacetic acid, 1 mm. below cut surface. Proliferated cells in endodermis, pericycle, and primary phloem matured as parenchyma; a few scattered tracheids (*tr*) in small, poorly organized vascular bundles. *B*, 0.002%, 0.5 mm. Extent of response unchanged from 3 days. *C*, 0.0002%, 0.5 mm. Stem tissues showing no response; maturation proceeding as in untreated material.

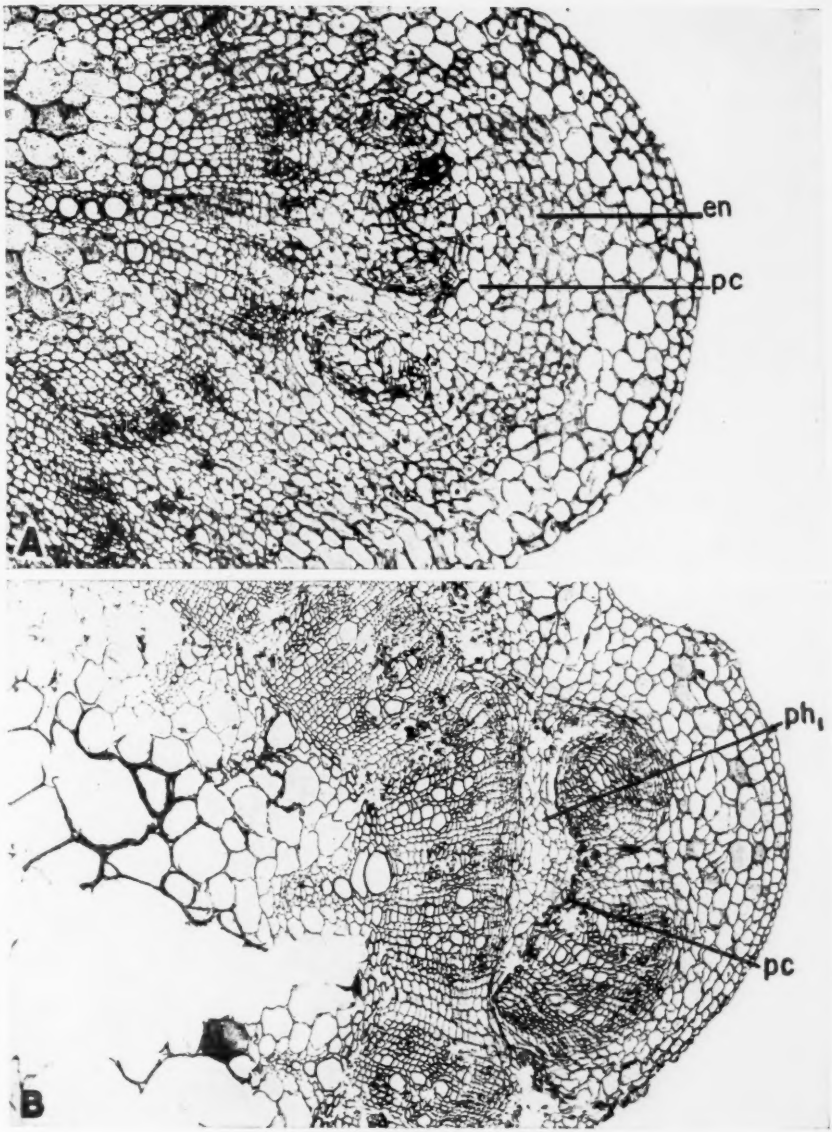

FIG. 14.—Transections of older stages showing position and character of vascularization. *A*, 14 days after treatment with 0.2% indoleacetic acid, 1.5 mm. below cut surface. Development of moderately large vascular bundles from derivatives of primary phloem parenchyma; proliferated endodermis maturing as parenchyma. *B*, 18 days after treatment with 2% tryptophane, at approximately 2 mm. Strongly developed bundles from derivatives of endodermis; no response in primary phloem parenchyma (ph_1). Compare compactly organized zone of secondary xylem with that in fig. 11. Disintegration of central pith characteristic for tryptophane.

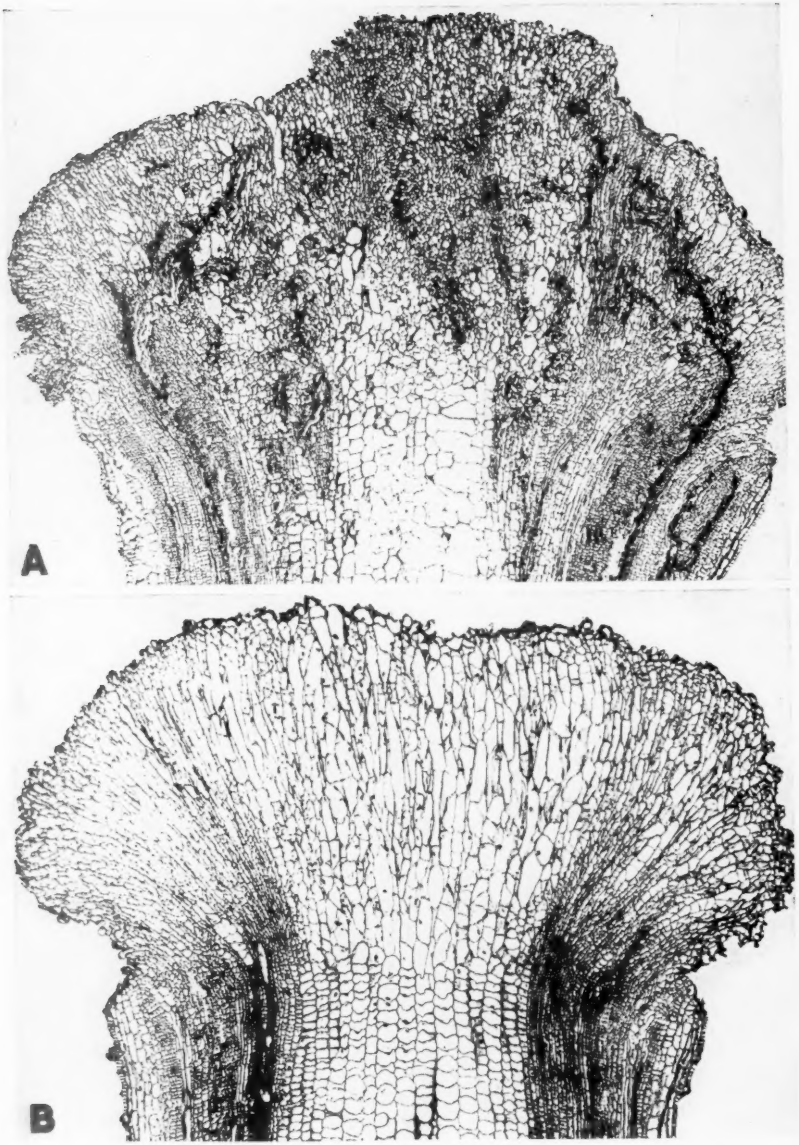

FIG. 15.—Longisections showing development of tumor above cut surface, 18 days after treatment. *A*, 0.2% indoleacetic acid. Cone-shaped tumor, largely derived from proliferation of pith; vascular tissues contributing to marginal area; complex system of vascular bundles showing connections with vascular cylinder of stem and vascular bundles proliferated in rays and pith. *B*, 0.02%. Smaller parenchymatous tumor, showing participation of pith as well as vascular tissues.

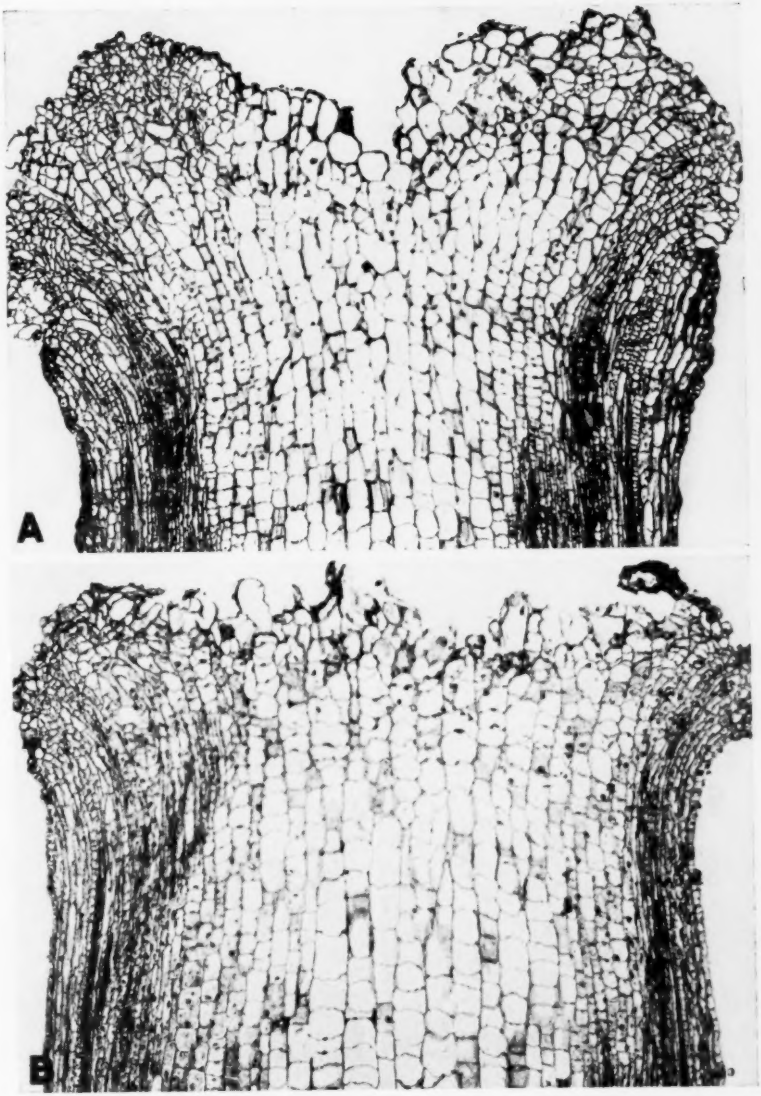

FIG. 16.—Longisections of stems treated with very low concentrations of indoleacetic acid. Limited proliferation at cut surface comparable with response in control material. *A*, 0.002%, 9 days after treatment. *B*, 0.0002%, 18 days after treatment.

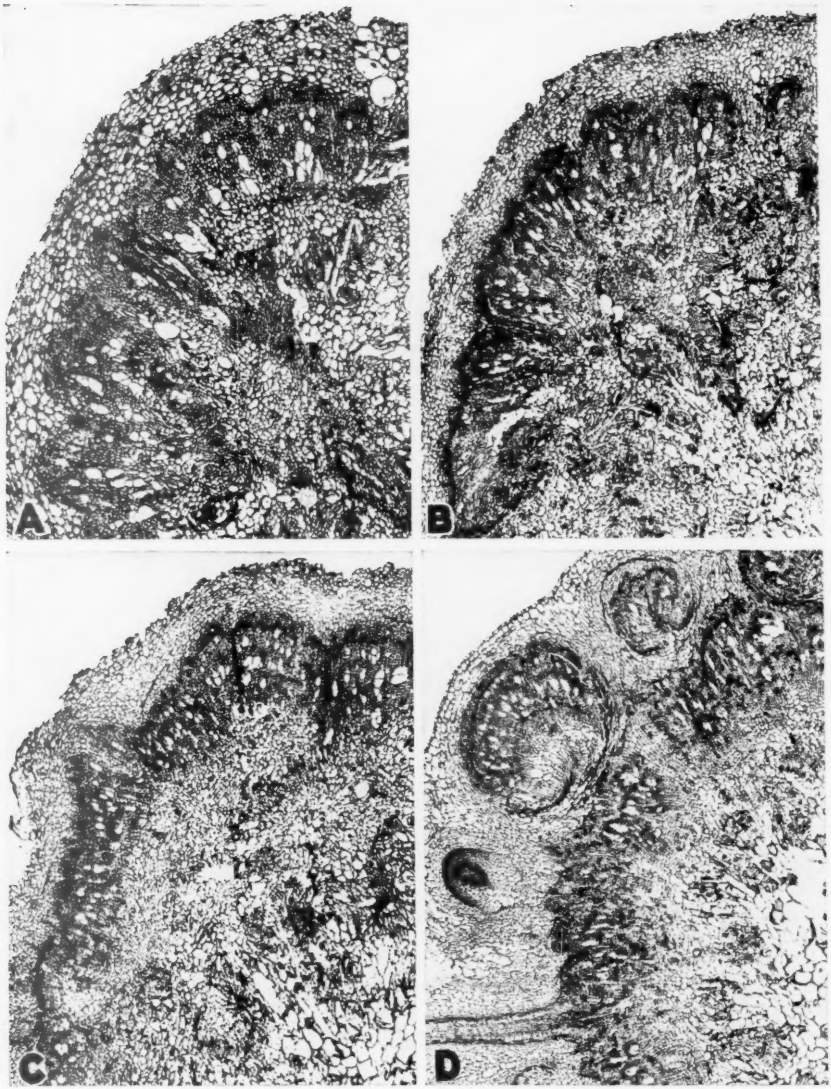

FIG. 17.—Transections of same stem, 18 days after treatment with 0.2% indoleacetic acid. Unusually large and highly vascularized tumor. *A*, 1.5 mm. above cut surface. Periphery meristematic; center highly vascularized with complex of anastomosing strands. *B*, 1 mm. above cut surface. Similar to *A*. *C*, 0.5 mm. above cut surface. Marginal band of vascular tissue, connecting at lower levels with endodermal bundles and vascular cylinder of stele; scattered small strands in center connect with bundles proliferated in pith. *D*, 0.5 mm. below cut surface. Characteristic organization of stem tissues evident; vascular bundles in endodermis, connecting at lower levels with stele; vascular strands from derivatives of pith centrad to protoxylem points.

The results of this study show that at none of the concentrations used did responses to indoleacetic acid duplicate those to tryptophane both in character and in rate of response. A comparison of the histological responses to various concentrations of indoleacetic acid and to tryptophane are presented in table 1. The important differences are as follows:

1. The cortical parenchyma is more reactive with tryptophane than with indoleacetic acid. (Compare fig. 6*D* with 6*B*.)

2. The degree of vascularization in the proliferated endodermis is greater with tryptophane. (Compare fig. 14*B* with 12*B*, 14*A*.)

3. The primary phloem shows little response with tryptophane; with indoleacetic acid it becomes not only active but also vascularized. (Compare fig. 14*B* with 14*A*.)

4. The course of vascularization in these outer tissues is characteristically different for the two substances. With tryptophane the endodermal bundles connect laterally through the outer ray with the stelar cambium and the respective secondary vascular tissues. With indoleacetic acid endodermal bundles pass across the pericycle into the primary phloem and at lower levels connect directly inward through a phloem ray with the cambium and the respective secondary stelar tissues. (Compare figs. 5*D*, 8*B*, 12*C* with figs. 9–11, 12*A*, *B*.)

5. With tryptophane the pith is relatively inactive and disintegrates in the center. It participates but little in the formation of the tumor, which consequently may show a depressed center. With indoleacetic acid the marginal pith actively proliferates and becomes vascularized. The central pith persists as a functional tissue and at the cut surface proliferates to form a considerable part of the cone-shaped tumor. (Compare fig. 1 of KRAUS [6] with our fig. 15*A*, *B*.)

These differences follow closely those found by Kraus in his comparison of the responses to the two substances, except that, as we have pointed out, the initiation of root primordia cannot necessarily be considered a critical point of difference between the effects of the two substances. Although there are certain points of similarity, the dissimilarities are sufficiently characteristic to suggest that the pattern of reactions resulting from the application of tryptophane is different from that upon application of indoleacetic acid, whether in high or low concentrations. On the basis of this histological individuality of effect, there appears to be a distinctive response attributable to tryptophane apart from any response which may result from the conversion of tryptophane to indoleacetic acid

In addition to histological differences between response to indoleacetic acid and to tryptophane, there are also differences in the time at which various reactions occur. The initial reaction in the endodermis was noted within 24 hours for all the substances described in table 1. In the first 3 days the degree of response to tryptophane, especially in the endodermis, resembles that of 0.02% indoleacetic acid (cf. fig. 2*B*, *D*). Maturation as made evident by differentiation of reticulate tracheids in proliferated tissues was noted by the 3d day with concentrations of 0.2% and 0.02% indoleacetic acid (cf. fig. 6*B*, *D*). However, maturation of vascular elements with tryptophane did not commence until the 6th day (fig. 8*A*, *B*). With concentrations of 0.2% and 0.02% indoleacetic acid, most of the proliferated endodermal cells were matured as parenchyma by the 6th day and with 0.002% by the 3d day. Meristematic activity seemed to continue in the endodermis through the 13th day with tryptophane. In other words, the response to tryptophane was just gaining

TABLE 1

HISTOLOGICAL EFFECTS OF TRYPTOPHANE AND VARIOUS CONCENTRATIONS OF INDOLEACETIC ACID ON BEAN

	Tryptophane (2.0%)	Indoleacetic acid			
		3.0%	0.2%	0.02%	0.002%
Response below cut surface:					
Epidermis	Ruptured at upper levels by expanding inner tissues	Ruptured by roots formed at upper levels	Ruptured by roots formed at upper levels	Remains intact	Remains intact
Cortical parenchyma	Active proliferation at uppermost levels	Outer cells much enlarged; inner cells highly meristematic	Outer cells much enlarged; inner cells meristematic	Cells slightly enlarged; a few divisions	No proliferation
Endodermis	Over major bundles broad arcs of proliferating cells; numerous bundles differentiated from derivatives	Broad band of intensely meristematic tissue, remaining active and participating in root formation; below root zone many derivatives differentiated as vascular tissue	Band of meristematic tissue; over rays derivatives active in root formation; over bundles derived cells matured as parenchyma or differentiated as vascular elements	Proliferated band 5–8 cells wide; slight vascularization	Slight proliferation over major bundles; two to five cells wide in places; matured as parenchyma
Pericycle	Slight response at cut surface; frequently crushed at upper levels	Slightly enlarged and becoming parenchymatous at upper levels; occasionally crushed	Similar to response to 3.0%	Similar to response to 3.0%	Little response
Phloem	Little response	Primary phloem parenchyma highly meristematic and similar to endodermis in root zone; at lower levels proliferation followed by vascularization; some proliferation of secondary phloem parenchyma	Primary phloem parenchyma generally meristematic in areas of root formation; otherwise moderately proliferated; well-organized vascular bundles at lower levels; little response in secondary phloem	Slight proliferation of primary phloem parenchyma; some vascular bundles at lower levels; otherwise inactive	Little response
Cambium	Increased activity	Rapidly increased activity	Increased activity	Increased activity	Slightly increased activity
Xylem	Wide band of compactly arranged secondary tracheids and vessels; medullary ray cells likewise differentiated as tracheids	Formation of moderately wide band of secondary tracheids and vessels with interspersed xylem parenchyma; parenchymatous medullary rays conspicuous	Less wide band of secondary tracheids with interspersed xylem parenchyma	Narrow band of secondary tracheids	A few secondary tracheids differentiated

TABLE 1—*Continued*

	Tryptophane (2.0%)	Indoleacetic acid			
		3.0%	0.2%	0.02%	0.002%
Outer ray	Active; derivatives typically form cambiums connecting vascular structures of endodermis and stele	Meristematic activity similar to primary phloem; forming roots at upper levels; maturing as parenchymatous cells at lower levels	Meristematic; participating in root formation or maturing as parenchyma	Slight activity associated with formation of interfascicular cambium	Inactive; interfascicular cambium infrequent
Inner ray	Inactive, except where derivatives of interfascicular cambium mature as tracheids	Highly active at upper levels, differentiating tracheids, clusters of sieve tubes, and vascular strands	Marked proliferation and differentiation of tracheids and vascular strands at upper levels; inactive at lower levels	Little activity	Inactive
Pith	Slight proliferation of parenchyma opposite xylem points; disintegration of central pith forms cavity	At upper levels activity in pith opposite xylem similar to that of inner ray; central pith remains active	Similar but less in degree than at 3.0%	Slight proliferation at upper levels	No proliferation but generally no disintegration of tissue
Root primordia	None	Numerous	Occurrence variable	None	None
Vascular pattern in outer tissues	Marked vascularization of endodermis with connections to stele laterally through rays	Organization and course of vascular bundles greatly confused by root formation; at lower levels large bundles occurring in endodermis and in primary phloem	Small vascular bundles; at upper levels mainly in endodermis; at lower levels some bundles end blindly, others cross pericycle into phloem and connect directly with vascular tissues of stele	Less well-organized vascular bundles, but following similar course as with 0.2%	None
Development at and above cut surface	At cut surface general proliferation of tissues except central pith; large tumor formed from derivatives of phloem, cambium, and xylem with periphery from endodermis; mainly parenchymatous with small vascular bundles	General proliferation of parenchymatous tissues at cut surface; very large tumor derived mainly from proliferation of pith and becoming highly vascularized	Similar proliferation of parenchymatous tissue at cut surface; very large tumor, derived mainly from proliferation of pith, but outer portions from proliferated xylem, cambium, and phloem; becomes highly vascularized	General proliferation at cut surface; tumor similar in derivation but smaller and less vascularized	General upward proliferation of tissues from phloem inward, forming small parenchymatous tumor

momentum at the time there was a slowing-down in the reaction to 0.02% indoleacetic acid, the concentration most nearly resembling tryptophane. Whether tryptophane is slow in inducing response or whether this retardation is related to a period of time necessary for the conversion of tryptophane to some unidentified growth-regulating substance remain problems.

Summary

1. The histological responses of decapitated bean stems to five low concentrations of indoleacetic acid were studied. Of these concentrations, the two highest—0.2 and 0.02%—induced marked histological changes in the stem and resulted in the formation of a distinct tumor above the cut surface. The intermediate concentration—0.002%—resulted in only slight histological response. The two lowest—0.0002 and 0.00002%—were apparently below the threshold of effectiveness for indoleacetic acid under conditions of this experiment. These last concentrations appeared to induce no greater response than that which occurs in control material. The essential features of the histological responses are summarized in table 1.

2. The responses shown for the three effective concentrations, as well as those described by KRAUS, BROWN, and HAMNER (7) for 3% indoleacetic acid, are essentially of the same pattern. In a stem tip treated with a high concentration, the responses at successively lower levels are similar to the responses which may be induced by treatment with lower concentrations.

3. A comparison of the patterns of response induced by indoleacetic acid and by tryptophane shows many dissimilarities. Significant differences have been described in the type of reaction, the degree of activity, and the vascularization of cortical parenchyma, endodermis, and primary phloem. The character and the degree of response in the pith and its relationship to tumor formation are likewise dissimilar. A more detailed comparison is presented in table 1.

4. At none of the concentrations used did both the character and the rate of response to indoleacetic acid duplicate in entirety the response to tryptophane. On the basis of this histological individuality of effect, there appears to be a distinctive response attributable to tryptophane apart from any response which may result from the conversion of tryptophane to indoleacetic acid.

Data and photomicrographs (figs. 2*D*, 5*D*, 6*D*, 8, 12*C*, and 14*B*) on 3% indoleacetic acid and 2% tryptophane are taken from other papers (**5, 6, 7**).

DEPARTMENT OF BOTANY
UNIVERSITY OF CHICAGO

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RESPONSES OF PTERIS LONGIFOLIA TO APPLICATIONS OF AMMONIUM 2,4-DICHLOROPHENOXYACETATE[1]

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 575

BERNARD STRICKLER

Introduction

Studies of the effects of 2,4-dichlorophenoxyacetic acid on plants with but one exception (**16**) have been confined to the responses of various angiosperms. Prior investigations have tended largely toward the refinement of techniques of applying this substance (**17, 12**) or an extension of its practical uses (**2, 11, 13**). The selective sensitivity to 2,4-dichlorophenoxyacetic acid of different species of plants, combined with the resultant telemorphic effects (**3**), has been of distinct advantage in its use as a herbicide.

In the present experiment information on the action of 2,4-dichlorophenoxyacetic acid is extended to the pteridophytes, especially to the gametophytic generation. The responses of the prothallia suggest the possibility of utilizing this compound as a tool of experimental morphology.

Material and methods

Spores were collected from a single leaf of *Pteris longifolia* grown in the greenhouses of the University of Chicago. Germination and subsequent culture of the gametophytes were carried out on new $2\frac{1}{2}$-inch unglazed clay pots which had been washed and then fired in an electric muffle furnace. The pots were then packed with white quartz sand which had previously been washed with distilled water and autoclaved. They were then inverted in small preparation dishes to which various solutions were added. When the entire pot became wetted, spores were sown on the topmost surface by tapping them from a dissecting needle. Enough solution was then added to submerge just the rim of the pot. The solutions were maintained at this level throughout the experiment by the addition of distilled water. The cultures were covered with transparent, untinted, cylindrical battery jars.

All solutions consisted basically of a one-half dilution of ZINZADZÉ's slightly acid formula (**18**), pH 6.1. Using this diluted nutrient solution, a stock solution containing 100 mg./l. of the ammonium salt of 2,4-dichlorophenoxyacetic acid was prepared. The ammonium salt was prepared by dissolving 2,4-dichlorophenoxyacetic acid in 95% ethyl alcohol, bubbling anhydrous ammonia gas into the above solution, washing the precipitate with absolute alcohol, and then drying. The final concentrations of the salt used in the experimental series were 50, 10, 1, and 0 mg./l.

The gametophytes received 10 hours of illumination daily from six 36-inch, 30-watt Mazda daylight fluorescent tubes set in a reflector (fig. 1). The temperature of the laboratory in which the prothallia were grown ranged approximately from 75° to 85° F.

Sporophytes used in the experiments were of two ages: small, young plants grown in pots, and large, mature plants growing undisturbed on a soil and rock substrate in the greenhouse. Their petioles and terminal pinnae were treated

[1] This work was supported in part by a grant from the Dr. Wallace C. and Clara A. Abbott Memorial Fund of the University of Chicago.

with concentrations of 2,4-dichlorophenoxyacetic acid ranging from 125 to 2000 p.p.m. These were applied either as lanolin mixtures of the acid or as aqueous solutions of the ammonium salt with Duponol C (9) as a spreader.

Gametophyte and sporophyte material prepared for microscopic examination was fixed in formalin-aceto-alcohol. Whole mounts of the prothallia were stained in Harris' haematoxylin, counterstained in orange G, and mounted in balsam. Sectioned material of the prothallia and sporophytes was prepared according to the tertiary butyl alcohol–paraffin method and stained with either the safranin–fast green combination or Flemming's triple stain.

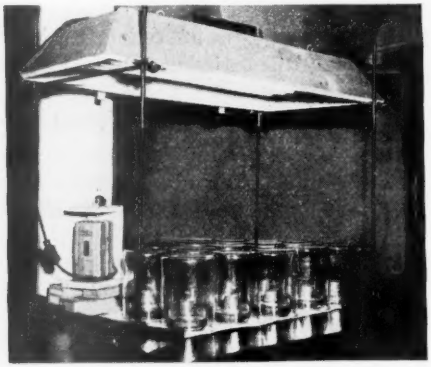


FIG. 1.—Arrangement of cultures of gametophytes under fluorescent lights.

Observations

SPORE GERMINATION

Throughout the course of the studies on the gametophytes, there was a high percentage of germination in all cultures. Nevertheless, in those cultures containing 10 and 50 mg./l. of ammonium 2,4-dichlorophenoxyacetate, a larger number of ungerminated spores were conspicuously present than in those containing plain ZINZADZÉ's solution or only 1 mg./l. of the salt. This led to the idea that the presence of this substance at the higher concentrations may have significantly reduced the percentage of germination. The larger size attained by the prothallia in the most dilute and control cultures, however, may have merely obscured the ungerminated spores, so that the visible differences might be more apparent than real.

To test this idea, a series of cultures was set up, according to the methods previously described, in which a number of 5 mm. squares were scratched into the surface of the pots on which the spores were to be planted. In each culture a combined total of 1000 ungerminated and germinated spores was counted. By confining the counts only to spores lying within the squares, the manner of selecting those to be counted was the same for all cultures.

In connection with the standardization of data on the percentage of germination, it should be noted that the significance of such data is affected by the differences in concepts of the definitive stage of spore germination. For example, ORTH (14) designated as germination that stage in development after the splitting of the exospore in which the first rhizoid or some other growth process is evident and chlorophyll is formed. Data based on counts of spores at earlier stages of development would fail in meaning for those who subscribe to ORTH's designation, since the number of plants which attain the requisite stage would be unknown.

Another factor of importance for this type of data is the time interval at which counts are made. DOPP (5) pointed out that the spores of a species of fern, even within the same culture, do not germinate at the same time. The successive

germination of spores of *Pteris longifolia* can be illustrated from our experiment, in which a preliminary count of 600 spores in a control culture revealed that the primary cell had emerged in 244 spores by the seventh day after sowing. Another count of the same area 2 days later showed 419 spores in the same or later stage of development.

For these reasons counts were made 10 days after the emergence of initial prothallial cells was first observed. All counts were made on the same day. Germinated spores then possessed more than one rhizoid and a number of prothallial cells. Ungerminated spores had failed even to split the exospore. The uniformity of germination among all the cultures (table 1) does not support the idea that germination was retarded either in extent or in time by the presence of ammonium 2,4-dichlorophenoxyacetate.

Gametophyte

Growth of the gametophytes was observed from whole mounts of prepared and fresh material collected at 3- or 4-day intervals. All stages of the treated plants were compared with untreated prothallia collected at the same times. Responses of gametophytes to the three concentrations of ammonium 2,4-dichlorophenoxyacetate used—though seemingly quite different at each concentration—showed two types of reactions which were fundamentally the same in all treated cultures: (*a*) an inhibition of growth in size and (*b*) abnormal patterns of development.

Inhibitory effects.—A difference in size was noted in gametophytes which were only 3 or 4 days old (figs. 2, 3). At this stage most plants possessed only one rhizoid; a few had two. At concentrations of 50 and 10 mg./l., the gametophytes were comprised of the single rhizoid and one or two prothallial cells, while those at 1 mg./l. and the control plants had filaments four or more cells long. As already mentioned, spores placed in nutrient solutions containing ammonium 2,4-dichlorophenoxyacetate were not delayed in germination so that this difference cannot be ascribed to age. That such is not the case is obvious from observations made on older gametophytes. Thirteen-day-old gametophytes at 50 mg./l. were still filamentous, consisting of about seven prothallial cells

TABLE 1

Results of tests of germination of spores of Pteris longifolia in ammonium 2,4-dichlorophenoxyacetate

Concentration of salt (mg./l.)	Ungerminated spores	Germinated spores	Total	Percentage of germination
0	257	748	1005	74.4
1	252	753	1005	74.9
10	247	756	1003	75.3
50	254	773	1027	75.1

and about six rhizoids (fig. 4). At all other concentrations the gametophytes were thalloid; the smallest being those grown at 10 mg./l.—the largest, the control plants (figs. 5, 6, 7). By the 27th day the untreated gametophytes had formed the typical heart-shaped prothallium; those at 1 mg./l. had begun to form a second lobe; the gametophytes at 10 mg./l. consisted of a single lobe, with no indication of forming a second one, while those at 50 mg./l. had remained essentially filamentous except for a small amorphous mass of cells near the apex.

Measurements of gametophytes when they were 3 and 27 days old showed that the size of prothallia grown at 50 mg./l. had increased only 3.39 times; at 10 mg./l., 48; at 1 mg./l., 113; while the control prothallia had increased 180

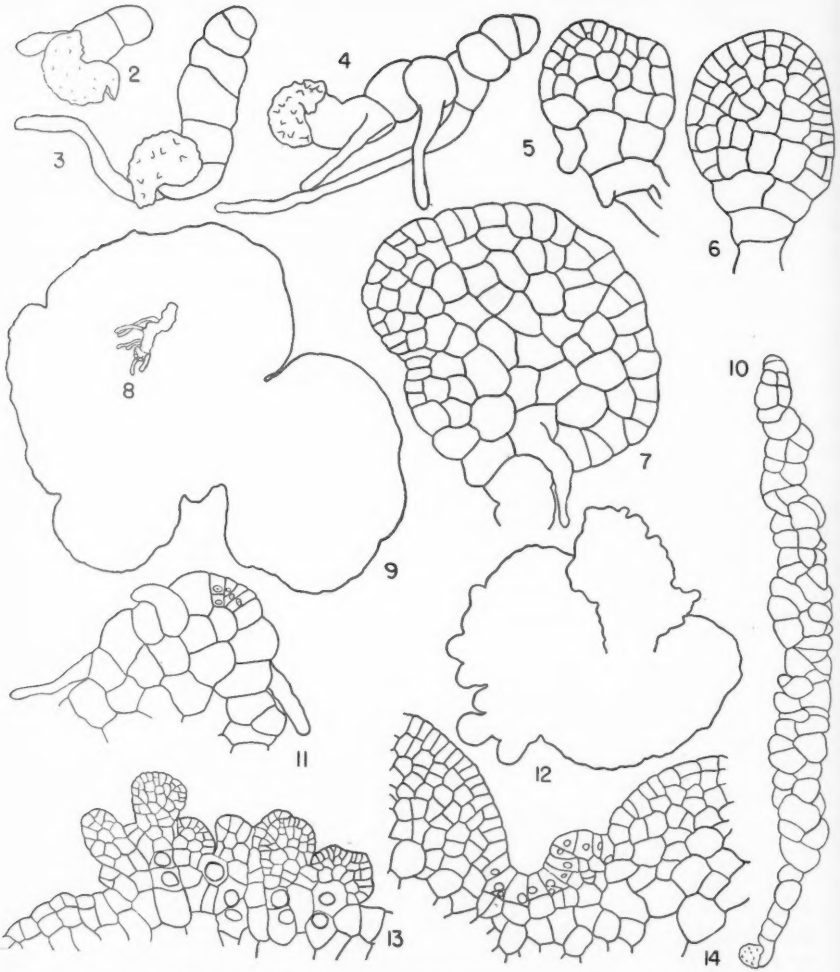


FIG. 2–14.—Gametophytes grown on various concentrations of ammonium 2,4-dichlorophenoxyacetate in ZINZADZÉ's nutrient solution (rhizoids mostly omitted). Figs. 2, 3, gametophytes 3 days old grown at 50 and 0 mg./l., respectively. Figs. 4–7, relative size of gametophytes 13 days old grown at 50, 10, 1, and 0 mg./l., respectively. In fig. 7 the meristem primordium is seen in typical lateral position on first lobe. Figs. 8, 9, relative size of gametophytes 30 days old at 50 and 0 mg./l., respectively. Fig. 10, gametophyte grown for 4 months at 50 mg./l., showing hemispherical end cell and juvenile filamentous condition. Fig. 11, apical portion of prothallium 27 days old, grown at 10 mg./l., with apical position of meristem on single thallus lobe. Fig. 12, prothallium 51 days old, at 1 mg./l., with vertical third wing and secondary prothallia on basal portion of thallus. Fig. 13, details of secondary prothallia of fig. 12. Fig. 14, early stage in development of third wing from base of sinus.

times. These values are based upon measurements of camera lucida drawings of the surface areas of the gametophytes. Each value represents the average of several gametophytes.

ALBAUM (1) has shown that, for fern prothallia composed essentially of a single layer of cells, surface area is a fair index of the mass of tissue present at any time and that increase in surface area may thus be used as an index of growth. Beyond the 27th day the prothallia in the present experiment developed more than a single layer of cells. After this, surface area was not used as an index of growth.

Differences in size between gametophytes of the same age grown at different concentrations of the salt were due chiefly to differences in cell number. The largest cells in the treated prothallia (figs. 4, 5, 6) were equal in size to those of untreated prothallia of the same age (fig. 7). This indicates that the two growth phases—cell division and cell enlargement—were dissimilarly affected by the experimental solutions. This is clearly shown in the prothallium in figure 5. Here the small number of larger, central cells composing the bulk of the thallus are surmounted by a marginal row of smaller cells of similar size, the "Randmeristem" described by ORTH. Intermediate-sized cells (fig. 7), which normally lie between the marginal meristematic cells and the more mature basal and central cells, are for the most part lacking, indicating that cell division but not cell enlargement was inhibited.

Striking evidence of the inhibitory action of the salt is seen in the comparison of 30-day-old gametophytes grown at a concentration of 50 mg./l. with control prothallia of the same age (figs. 8, 9).

FORMATIVE EFFECTS AT 50 MG./L.—There was no evidence of plate formation in gametophytes grown at 50 mg./l. until 30 days after germination. Until this time they consisted of a linear series of cells always derived from divisions of the hemispherical terminal cell. The filament was distorted, owing to the unequal distension and consequent bulging of the wall of each maturing cell. Except for the formation of rhizoids, divisions of these derivatives were rarely seen. After the 30th day subsequent derivatives of the end cell divided in an irregular manner. Ultimately, an elongated clump of distended cells was formed, comprising the only "thallus" seen in these gametophytes throughout the experiment. From the small size of the cell mass, attained over a period of 4 months, it is unlikely that the lineage from each derivative of the end cell was more than a single cell (fig. 10).

The appearance of these retarded gametophytes is unlike that commonly described for gametophytes whose juvenile stage has been prolonged by subjection to a variety of other abnormal conditions, particularly those of light (**4, 10**). These latter filaments are almost always attenuated, branched, and usually produce antheridia. None of these characteristics was seen in the present gametophytes; they formed no sex organs, were never branched, and the cells, as already indicated, were not attenuated.

GAMETOPHYTES GROWN AT 10 MG./L.—These gametophytes showed two interesting types of peculiarities, the first of which concerns the position of the meristem. Although every gradation between the apical and lateral position of the meristem primordium is found among ferns with cordate prothallia (**6**), the prothallium of *P. longifolia* typically develops from a lateral meristem.

Development of the untreated gametophytes conformed to the usual type of

development of *P. longifolia* as described by GOEBEL (**6**, p. 204): "The surface of the prothallus which first develops from the germ-thread becomes at once the one wing, and the meristem which forms the vegetative point of the prothallus comes thereby to occupy a lateral position, and underneath it the second wing of the prothallus shoots out. Figure 150 exhibits this process in *Pteris longifolia*.[2] Here it will be seen that a one-layered cell surface is formed first of all from the germ-thread without the aid of an apical cell, and the anticlinal walls diverge at the apex. . . . . The intensity of the cell multiplication remains strongest at a lateral position on this cell surface, and there is the meristem in which often a two-sided apical cell is visible." In all the untreated plants observed in the present study, the meristem always arose laterally on the first formed wing (fig. 7).

Gametophytes treated with 10 mg./l., on the other hand, consistently formed their meristem apically. In this respect, these prothallia conformed to similar stages described for *Pteridium aquilinum* (**14**) and *Onoclea struthiopteris* (**5**, **14**), in which the meristem typically lies in the longitudinal axis of the prothallium (fig. 11).

Because of the slow rate of cell division in treated gametophytes, the segments formed on either side of the meristem failed to extend beyond the vegetative point; thus, these prothallia did not become heart-shaped (fig. 15).

The second peculiarity was observed in prothallia 51 days old, on which a thick cushion consisting of distended, loosely arranged cells covered most of the ventral surface. In nearly all prothallia a prominent, compact tubercle was growing within the cushion close to the apex. A few prothallia had two tubercles lying side by side in the same forward position. In other prothallia these masses projected above the dorsal surface (fig. 15), but for the most part they were confined to the ventral surface.

The tubercles consisted of a large number of small, densely cytoplasmic cells with large nuclei (fig. 16). The cells did not exhibit any correlation but appeared to have divided at random. The entire cell mass was inclosed by the large vacuolate cells of the cushion.

Nearly all prothallia observed were without sex organs. Rarely, one or two unopened archegonia were seen on the cushion.

GAMETOPHYTES GROWN AT 1 MG./L.—Increase in size at this concentration also proceeded at a slower rate than in plain ZINZADZÉ's solution so that at the time of sex-organ formation these prothallia were considerably smaller than the control plants. Up to the 30th day, the gametophytes followed the usual pattern of development and attained the cordate shape. Subsequently, the cushion in the treated plants became more massive and extensive than that formed by the controls. Sex organs which later appeared on the cushion were more numerous on treated plants; especially antheridia.

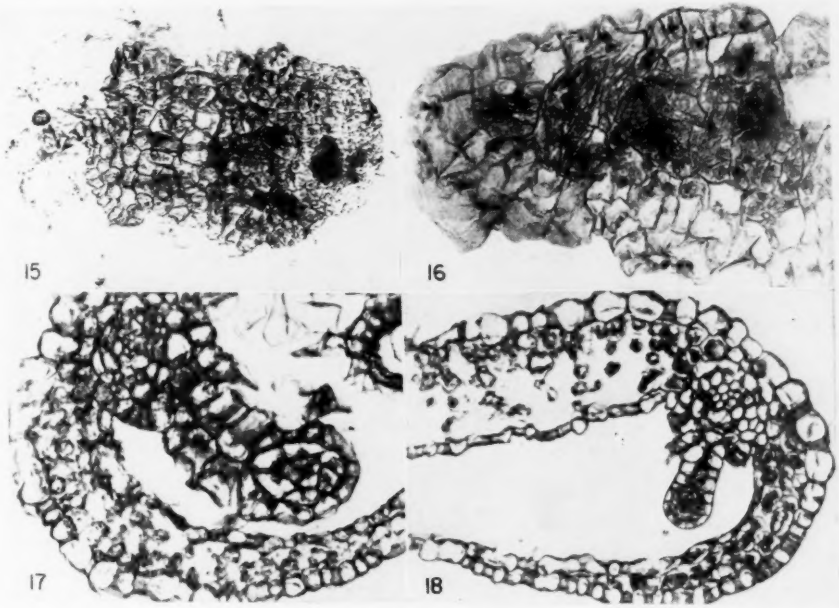
In plants treated at the 1 mg./l. concentration of ammonium 2,4-dichlorophenoxyacetate, there followed next renewed vegetative activity of the apical meristem, first evidenced by a small, tongue-like projection lying close to the base of the sinus formed by the two wings (fig. 14). Elongation of the projection proceeded in an upward direction accompanied by rapid lateral expansion. Thus, in 51-day-old gametophytes the meristem was borne at the apex of a third wing, usually fan-shaped with undulating margins (fig. 12), but some-

[2] Figure 7 of the present paper shows an equivalent stage to fig. 150 of Goebel.

times with margins curled to form a cylinder.

The vertical position of this wing permitted illumination on both sides so that it was not surprising to find sex organs on both surfaces. In addition, archegonia were present on the margin of the apex on either side of the meristem.

process were observed, but, by tracing cell derivatives, it is clear that marginal cells became meristematic, forming a plate directly, with no intervening filamentous stage (fig. 13). Owing to the simultaneous activity of many marginal cells, the adventitious thalli were crowded.



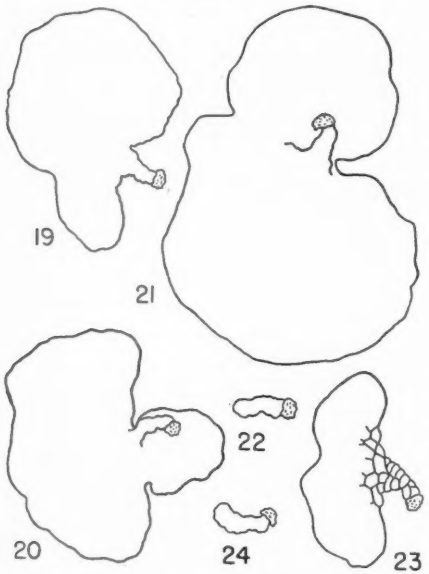
FIGS. 15–18.—Fig. 15, prothallium grown at 10 mg./l. solution of ammonium 2,4-dichlorophenoxyacetate, showing tubercle in apical region. Fig. 16, longisection of tubercle showing random arrangement of small, densely cytoplasmic cells. Fig. 17, transverse section of pinna treated with lanolin mixture of 500 p.p.m. 2,4-dichlorophenoxyacetic acid, showing grossly enlarged sporangium and receptacle. Fig. 18, pinna of control.

Simultaneously with the formation of the third wing in 51-day-old gametophytes, secondary prothallia were growing from the basal portion of the old wings (figs. 12, 13). Each of these new growths was intimately attached to the old gametophyte by means of a broad base, cells of which evidently were part of the parent tissue. Many of these older cells bore antheridia. No initial stages of this regeneration

Sex organs on plants treated with 1 mg./l. of ammonium 2,4-dichlorophenoxyacetate were functional, young sporophytes appearing on most prothallia in 120-day-old cultures. No irregularities were observed on primary leaves, and such plants were transplanted to agar in Petri dishes where they grew well for 4 months and gave every indication of continuing vigorous growth. In all respects these young sporophytes were

comparable to those formed at the same time on control prothallia.

TRANSPLANT EXPERIMENT.—Figures 19–24 show the results of experiments made to determine (*a*) the effect of 50 mg./l. of ammonium 2,4-dichlorophenoxyacetate on young prothallia not previously treated and (*b*) the permanency of the effect on gametophytes germinated and grown for a period of time on this same substance. The methods were the same as those previously described, except that the spores were sown on squares of glass cloth placed on the upper surface of inverted pots instead of directly on the pot surface. In this way prothallia were easily transferred from one pot to the other.



FIGS. 19–24.—Fig. 19, prothallium 21 days old grown from spore on plain nutrient solution. Fig. 20, prothallium grown for 21 days on plain nutrient solution, then transferred and grown for 12 days on 50 mg./l. of ammonium 2,4-dichlorophenoxyacetate. Fig. 21, prothallium grown continuously on plain nutrient solution for 33 days. Figs. 22–24, reciprocal of experiment shown in figs. 19–21.

Figure 19 shows a prothallium which was grown for 21 days on plain ZINZADZÉ's solution. A number of prothallia of this age were then transferred to the cultures containing 50 mg./l. of ammonium 2,4-dichlorophenoxyacetate. After 12 days these gametophytes (fig. 20) were compared with those of the same age—now 33 days old—which had not been transferred (fig. 21).

It is readily seen that the retardation of cell division is not a response in which the initial treatment of the germinating spore is a necessary condition but that the inhibitory action of ammonium 2,4-dichlorophenoxyacetate is similarly effective on later stages of prothallial development.

In the reciprocal experiment, spores were germinated and grown on cultures containing 50 mg./l. of the salt. After 21 days the minute filaments (fig. 22) were transferred to plain ZINZADZÉ's solution. Within 3 or 4 days the apex of the gametophytes had broadened considerably, and at the end of 12 days the two wings were well established (fig. 23). During this 12-day period the gametophytes which had remained in contact with the nutrient solution containing the growth-regulating substance showed no appreciable increase in size (fig. 24).

Renewed meristematic activity of transplanted prothallia was also observed in the tuberculate plants resulting from the 10 mg./l. treatment. A number of these gametophytes were transplanted to agar plates in an effort to induce further growth of the tubercles. Instead, germinal filaments soon emerged from marginal and inner cells of the thallus. These filaments formed the usual spatulate lobe, developed a meristem, and became cordate. The original prothallia died.

It is apparent from the regenerative growth of the transplanted prothallia

that the inhibitory activity of ammonium 2,4-dichlorophenoxyacetate does not persist once the plants are removed from actual contact with the effective solution. This behavior is not wholly unexpected when one considers that each cell of the thallus is potentially capable of reproducing a new prothallium and that the amount of the substance in a single cell at any one time must be exceedingly small. The derivatives of such a cell, having once succeeded in dividing, would then rapidly become removed from the influence of the residual substance.

Sporophyte

Applications of lanolin mixtures and aqueous solutions were made at the basal portion of the petioles, usually below the first pinna, at the time the leaves had unfolded about one-half their ultimate length. The mature plants at this time were producing leaves 40 cm. or more in length, while the leaves of the young potted plants were rarely 15 cm. long. Applications on the mature plants were at 500 and 1000 p.p.m.; the young plants, in addition to these concentrations, were treated with 125, 250, and 2000 p.p.m.

Gross reactions.—Within 24–48 hours after treatment the petioles at various distances from the point of application showed pronounced downward bending, except at a concentration of 125 p.p.m. at which there was no reaction. As the croziers slowly unrolled, the petioles continued bending in one direction or another until the full length of the leaf was attained. In all cases the final length was less than that of the controls.

Reactions of the pinnae were primarily characterized by a failure to unfold completely (fig. 25*d*). For a period of 3 or 4 weeks after treatment the pinnae slowly continued to unroll and expand, after which they persisted in one or another of the stages of unfolding. Retardation in this respect was greatest in the uppermost (i.e., the youngest) pinnae and decreased basipetally to the pinnae which were fully expanded at the time of treatment. These latter pinnae exhibited no effects from the treatments.

Intensity of responses appeared to be related more to the relative age of leaves at the time of application than to concentrations used. In preliminary experiments numerous applications were made on leaves of similar ages, and the intensity of responses showed considerable variation in which the most marked reactions were not always associated with the highest concentrations used (figs. 25*a*, *b*, *c*). Responses were more rapid to aqueous solutions than to the lanolin mixtures.

Histological reactions.—Several days after the curvatures of the petioles appeared, the leaves became unusually inflexible, the young pinnae and young portions of the petioles acquiring a brittle quality. Although mature petioles of *P. longifolia* possess a natural toughness because of the thick-walled epidermis and the several layers of thickened outer cortical cells, newly differentiated parts of the petiole are supple.

Transverse sections of these young parts showed that the subepidermal layers of the cortex were already considerably thickened, hence more mature than comparable tissues of the control plants. Tertiary thickening of the metaxylem elements also occurred earlier in the treated plants, but this was evident only in a small number of elements surrounding the invaginated phloem in the arms of the horseshoe-shaped stele. The most pronounced thickening of the vascular elements occurred in the treated basal portions of petioles of mature

plants; especially where the aqueous solution of ammonium 2,4-dichlorophenoxyacetate was used.

Aside from the early maturation of these tissues, and a slightly greater thickening of all the cortical and stelar tissues, no other histological irregularities of the petioles were evident. There were no secondary outgrowths, tumors, proliferations, or derangements in the position of the tissues.

THE SPOROPHYLL.—The terminal pinnae, from which the observations of the reactions of the sporangium were made, were treated when the pinnae were completely unfolded and the indusium was still closed. By the use of a hypodermic syringe, a thin, uniform thread of the lanolin mixture of the acid (500 p.p.m.) was laid along the indusium on one side of the pinna. The indusium on the other side received no treatment. On a second pinna a thin band of the aqueous solution of ammonium 2,4-dichlorophenoxyacetate (500 p.p.m.) was painted on one indusium with a camel's-hair brush. These treatments were repeated on several plants.

Within 2 days after application there was a marked epinasty of the treated

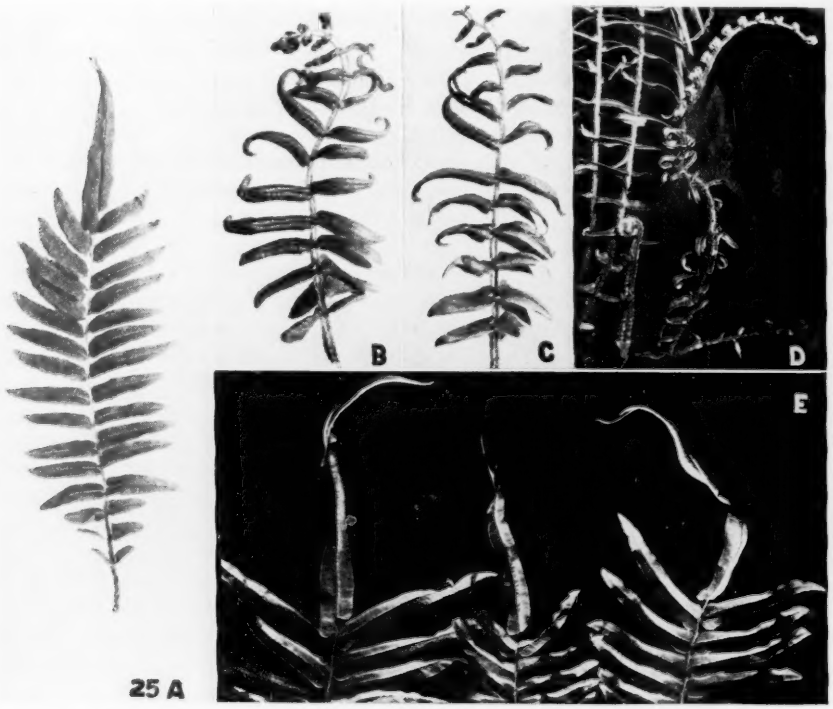


FIG. 25.—Fig. 25*A–D*, leaves 30 days after treatment with ammonium 2,4-dichlorophenoxyacetate: *A*, control; *B*, 500 p.p.m.; C, 2000 p.p.m.; *D*, 1000 p.p.m., leaf of mature plant showing marked failure of pinnae to unroll. Fig. 25*E*, terminal pinnae 14 days after unilateral treatment: *left*, lanolin mixture of 500 p.p.m. 2,4-dichlorophenoxyacetic acid; *middle*, lanolin control; *right*, aqueous solution of 500 p.p.m. ammonium 2,4-dichlorophenoxyacetate.

halves of the pinnae. By the 4th day the pinnae treated with the aqueous solution showed a spiral twisting along the axis of the treated indusium. Similar but less pronounced responses followed the application of the lanolin mixture. In preliminary experiments pinnae so treated persisted in the twisted condition for an indefinite time without further gross responses. The present material, however, was collected at the end of 14 days (fig. 25*e*).

Histologically, the pinnae were little affected by the growth-regulating substance. Transections showed enlargement of cells of the upper epidermis associated with the curvatures on the treated side of the blade, and there was also some derangement of the palisade layer accompanying this same response.

In contrast to the inactivity of the pinna in general, the quantitative reaction of the sporangia and receptacle was very striking. It was characterized by pronounced cell enlargement which resulted in the growth of these structures to more than twice their usual size.

The receptacle of *P. longifolia* is not prominent, being only slightly elevated above the plane of the lower epidermis. The receptacle beneath the treated indusium, however, became a rather massive structure which protruded well beyond the epidermis (fig. 17). The receptacle on the untreated side of the pinna was relatively unaffected (fig. 18).

A comparison of figures 17 and 18 also shows that the sporangia beneath the treated indusium were markedly affected and that the cells of these sporangia, particularly those of the stalk and tapetum, exhibited the same swollen condition as the receptacular cells. Although the sporangia and receptacles are meristematic up to the time the indusium is fully open and the sporangia are mature, these organs were not stimulated to proliferate. The number of cells comprising the receptacle, and especially the sporangia, were always consistent with the particular stage of development in which they were observed.

Discussion

The results of the experiments on the gametophytes show that the primary and general effect of ammonium 2,4-dichlorophenoxyacetate was inhibition of growth. This effect was essentially the result of the retardation of cell division with little or no effect upon cell enlargement. In contrast to the behavior of the sporophytes, the quantitative responses of the prothallia were directly correlated with the concentration of the effective substance. Thus, at 50 mg./l. retardation was so great that no plate was formed, whereas at 1 mg./l. the gametophyte developed into a heart-shaped prothallium with functional sex organs.

Aside from the obvious inhibitory effect on growth of the gametophytes, it is less apparent whether the unusual structures observed on the prothallia were the result of the direct influence of ammonium 2,4-dichlorophenoxyacetate or whether these growths were secondary responses resulting from other causes.

For example, it is not certain whether the tubercles which appeared on prothallia grown at 10 mg./l. were apogamous embryos whose differentiation was unorganized due to the influence of the growth-regulating substance or whether they were gall-like masses resulting from proliferation of the cushion cells.

HEIM (**8**) has described certain projections from the cushion of *Doodya caudata* from which apogamous sporophytes arose, and the internal appearance of these structures as figured by him bear a resemblance to that of the present tuber-

cles. In the case of *Doodya*, however, the sporophytic nature of the projections was confirmed at a relatively early stage by the development of tracheids, later followed by the formation of the apices of the leaf, stem, and root of the embryo, which then grew in a normal manner.

Usual evidences of apogamy in ferns (15), such as the formation of tracheids, or hairs surrounding the embryo, were not present in the treated prothallia, and the cell masses as seen in longitudinal and transverse sections were not recognizable embryos. In addition, transplants made to induce further growth of the tubercles failed. It does not appear likely that the tubercles were sporophytic.

The renewed activity of the apex of prothallia grown at 1 mg./l. also suggests the possibility of secondary formative influences. It is well known that the gametophytes of the leptosporangiate ferns are much more plastic than the sporophytes and that relatively minor alterations of the environment of the prothallia induce profound form changes.

In this connection it is interesting that the outgrowth of the apex in the treated prothallia is strikingly similar to that obtained by ALBAUM (1) in young prothallia which were changed from their nearly vertical position to a horizontal one. Following the reorientation, the apex in these prothallia began to grow upward toward the source of light, and there was ultimately formed a third wing similar to that shown in figure 12. It is not to be inferred, however, that the apical outgrowths in the treated prothallia necessarily originated in this same manner, since their position relative to the source of light was not experimentally altered.

The curvatures of petioles and pinnae following treatment of the leaves of *P. longifolia* indicate that the reactions of the sporophyte of this fern to 2,4-dichlorophenoxyacetic acid are not markedly different from those of various angiosperms that have been similarly treated.

In the first report of the activity of this substance on plants, ZIMMERMAN and HITCHCOCK (17) designated as a general type of response "the negative curvatures of stems and leaves, i.e., responses involving cell elongation." Subsequent studies by these investigators and others have shown that distorted growth of stems and leaves is readily induced in many angiosperms by applications of 2,4-dichlorophenoxyacetic acid and is one of the first conspicuous effects of such treatments. In the same paper, ZIMMERMAN and HITCHCOCK described a second type of effect—"morphogenetic activity." This term was used to refer to "modifications of organs which involved responses other than curvatures due to cell elongation." Except for the modification of sporangia, which was basically the result of cell enlargement, this second type of response was strikingly absent in the treated sporophytes. The tissues of the leaves were relatively unaffected by the applications of 2,4-dichlorophenoxyacetic acid or its ammonium salt and were quite insensitive to the formative influences of this substance. The resistance of the sporophyte of *P. longifolia* to histological modification when treated with 2,4-dichlorophenoxyacetic acid is not unique but serves as another example emphasizing the selective action of this substance.

Summary

1. Spores of *Pteris longifolia* were germinated and the gametophytes were grown uninterruptedly with ZINZADZÉ's

solution containing 0, 1, 10, and 50 mg./l. of ammonium 2,4-dichlorophenoxyacetate.

2. Spore germination was not adversely affected either in time or in percentage by the presence of the growth-regulating substance.

3. The smaller size of treated gametophytes was due to the smaller number of cells comprising them and not to the size of the cells. The intensity of retardation of cell division was proportional to the concentration of the ammonium salt used.

4. Treatment at 10 mg./l. resulted in the formation of the meristem primordium in the apical instead of the usual lateral position. These prothallia did not become heart-shaped and rarely produced sex organs.

5. Fifty-one-day-old gametophytes growing at 10 mg./l. exhibited tubercles consisting of small, densely cytoplasmic cells in random division within the massive cushions.

6. Prothallia growing at 1 mg./l. showed renewed vegetative activity of the meristem to form a third wing. Simultaneously, secondary prothallia were growing from the basal portion of the old prothallial wings. Functional sex organs were more abundant on the prothallia grown at this concentration than on the controls.

7. Young prothallia grown for 21 days on plain ZINZADZÉ's solution made no appreciable growth after being transferred to a culture containing 50 mg./l. of ammonium 2,4-dichlorophenoxyacetate. Conversely, gametophytes which were germinated and grown for 21 days on the latter culture showed a rapid recovery to normal growth after being transferred to plain ZINZADZÉ's solution. Tuberculate prothallia transferred from 10 mg./l. cultures to agar plates quickly produced secondary prothallia which were normal in all respects. The tubercles made no further growth.

8. Leaves of young and old sporophytes of *P. longifolia* were treated with lanolin mixtures of 2,4-dichlorophenoxyacetic acid (500 p.p.m.) and aqueous solutions of the ammonium salt of the acid (125–2000 p.p.m.). The petioles showed marked curvatures and the unrolling of pinnae was retarded. These responses were permanent. There was no reaction from applications of 125 p.p.m.

9. Histologically, the applications resulted in earlier maturation of the petioles, especially in the outer cortex and the metaxylem elements surrounding the invaginated phloem. There were no tumors, proliferations, or derangement of the tissues. The responses were more marked where the aqueous solutions were used.

10. Unilateral treatment (500 p.p.m.) of terminal pinnae resulted in epinasty and spiral twisting of these organs. Beneath the treated indusium, the receptacle and sporangia were grossly enlarged due to cellular distension. There was no unusual increase in the number of cells comprising these structures.

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DEPARTMENT OF BOTANY
UNIVERSITY OF CHICAGO

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THE EFFECT OF INDOLEACETIC ACID UPON INITIATION AND DEVELOPMENT OF HYPOCOTYLEDONARY BUD PRIMORDIA IN FLAX

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 576

GEORGE K. K. LINK AND VIRGINIA EGGERS[1]

Introduction

Generally, the normal nondecapitated hypocotyl of *Linum usitatissimum* develops neither buds nor a shoot. If it does, the buds and shoot are restricted to the base of the hypocotyl (**2, 6**). If decapitated at a suitable time, however, the hypocotyl generally develops buds abundantly at all levels and, in time, a shoot (generally, one only completes development) which substitutes for the lost axis (**2**). In the second paper of this series, LINK and EGGERS (**6**) reported that, while bud primordia are initiated in the lower half of the intact hypocotyl of flax, none are initiated in its upper half. Following decapitation, however, the upper half, too, initiates bud primordia, and the lower half probably initiates some in addition to those present before decapitation.

[1] This is the third of a series of papers reporting the results of studies of hypocotyledonary and epicotyledonary vegetative buds and wound tissues of *Linum usitatissimum* by GEORGE K. K. LINK and VIRGINIA EGGERS which have extended from 1934 to date. The work was supported in part by a grant from the Dr. Wallace C. and Clara A. Abbott Memorial Fund of the University of Chicago.

The study reported here was undertaken on the assumption that the cotyledons and epicotyl have an inhibitory effect on the initiation and development of hypocotyledonary bud primordia in flax and that this effect is brought about by an auxin or by a complex involving one or more auxins in proper concentration. The decapitated hypocotyl of flax, accordingly, was used to test the effect of indoleacetic acid in lanolin upon the formation and development of hypocotyledonary buds when applied to the decapitation cut. The primordium of a hypocotyledonary bud of flax is initiated by transverse division of an epidermal cell without special prior enlargement or increase in protoplasm of the cell about to divide (**5, 6**). Therefore, it seemed that this study might test the effect of indoleacetic acid upon division of a cell—without prior enlargement of the cell—as manifested in the initiation and in the early stages of development of a hypocotyledonary bud primordium in the upper half of the hypocotyl, as well as upon the complex of cell enlargement, cell division, and cell differentiation manifested in subsequent development and growth of the young bud into a shoot.

Material and methods

Seed of the Bison strain of *L. usitatissimum* was planted in quartz sand in 6-inch pots watered with the complete mineral nutrient solution used by EGGERS (**3**). The plants were grown in the greenhouse, and plantings were made throughout the year. Seedlings were thinned so as to leave 20–25 plants per pot. Some plants were left as uncut controls, but most were decapitated directly below the cotyledons, generally on the 10th day after emergence of the seedlings from the substrate. The necessary number of decapitated plants was used as untreated cut controls, and the others were used for treatment with lanolin alone or with indoleacetic acid in lanolin in various concentrations. The concentrations tested ranged from 3% indoleacetic acid to 0.00000012% (4.5×10^{-7} molal). In preparation of concentration 0.116% indoleacetic acid (6×10^{-3} molal and designated 1:1 in this paper), one part of saturated aqueous solution of indoleacetic acid was stirred into an equal part of anhydrous lanolin. From this standard mixture higher dilutions were prepared by addition of proper amounts of lanolin. The lanolin alone and the lanolin–indoleacetic acid mixtures were applied to the cut surfaces of the hypocotyls in sufficient quantity to cap the stumps. In some experiments only one application was made; in others they were made daily.

In experiments 1–5, one pot each with 20–25 plants was used as a test lot, and the plants were observed daily. Readings were made at definite intervals in terms of the number of plants budded and of the number of buds per budded plant. Results of the former readings are expressed as percentages of plants budded per test lot; results of the latter, as the average number of buds per plant of each test lot.

Variability of individual plants as to bud formation and development was found to be so great that data were required from which the standard error (SE) and the value of t could be calculated. To this end experiments 6 and 7 were set up.[2] In experiment 6 each test lot consisted of five pots, each with twenty plants, and in experiment 7 each test lot consisted of six pots, each with

[2] We are indebted to our colleague Professor SEWALL WRIGHT for counsel and advice in setting up these tests and for the equations used to determine SE and t.

twenty plants. The value of SE was calculated according to the equation

$$SE = \sqrt{\frac{(\Sigma V_1^2 - \overline{V}_1 \Sigma V_1) + (\Sigma V_2^2 - \overline{V}_2 \Sigma V_2)\left(\frac{1}{n} + \frac{1}{n}\right)}{2n - n}},$$

in which V is the average number of buds per plant in each pot of each test lot; n is the number of pots in each test lot; ΣV is the sum of the averages of the pots of each test lot; $\overline{V} = \Sigma V/n$ is the average of the averages (V) of the pots of each test lot; ΣV^2 is the sum of the squares of the averages of each pot of a test lot; and $\overline{V}\Sigma V$ is the product of $\overline{V}$ and ΣV. The value of t was calculated according to the equation

$$t = \frac{\overline{V}_1 - \overline{V}_2}{SE}.$$

The values of SE and t were determined for various pairs of test lots from the data of the next to the last reading of experiments 6 and 7. Detailed data are given in table 3 and figures 2 and 3 for experiment 7 only; in this experiment the differences noted in bud averages per test lot were great enough to be statistically significant.

Counts were made only of the grossly detectable buds. If they had been made on sectioned material with a microscope, the values for buds present would have been higher and the intervals after treatment until buds were first recorded would have been shorter.

Samples of hypocotyls were collected, fixed, imbedded, sectioned, and stained according to the methods reported in our earlier paper (6). The sections were studied to determine the effects of the application of lanolin and of indoleacetic acid in lanolin on the behavior of epidermal cells of the upper half of the hypocotyl with reference to the cell divisions which initiate bud primordium formation, and upon the development of primordia present in the lower half of the hypocotyl at the time of decapitation and treatment.

Observations and findings

In the decapitated hypocotyl of flax the initial gross injury (the cut) is succeeded by the following train of injury effects: the decapitated member generally guttates for a period whose length depends upon the physiologic state of the hypocotyl prior to decapitation and upon external conditions following it; it shrinks, evident in decrease of its diameter and, at times, even of its length; it becomes less green; the cut surface begins to dry out and becomes necrotic, the depth to which the underlying tissues are involved depending upon the physiologic state of the hypocotyl prior to the primary injury and upon external conditions following it. In vigorous hypocotyls recovery from the injury and injury effects attendant to decapitation is marked by prompt reattainment of the green color and of the diameter of the predecapitation hypocotyl; by early cessation of desiccation and necrosis of the cut and of the tissues below it; by prompt cicatrization of the wound; and by slight tumor formation immediately below the healing surface through development of an inner wound tissue (interior callus). After this the hypocotyl experiences a limited general diameter increase, and, in time, buds become grossly evident on most hypocotyls (fig. 1*D*).

If a hypocotyl succeeds in healing the wound promptly and develops a shoot, this shoot generally attains only one-half

the length of the main axis of a nondecapitated plant, but its blooming lags only a few days behind that of the nondecapitated plant. Since such a plant does not develop the cotyledonary axes of the normal plant, even in recovery it still is abnormal and pathic in that its production of the fiber-bearing members and its production of flowers and fruit are far behind that of the normal plant. The primary injury and its effects leave a lasting, irreversible effect even under the most favorable circumstances.

If the hypocotyls are less vigorous when decapitated and external conditions are not favorable, the decapitation injury and its effects may terminate fatally in many hypocotyls. The older the hypocotyl at time of decapitation, the greater is the chance of death of the pathic hypocotyl. Hypocotyls of plants grown in soil are more pathic and die more readily than those grown in sand and supplied with mineral nutrients. The pathic state of a decapitated hypocotyl may be brief, ending promptly in death, or prolonged, terminating in death only after some weeks. Development of hypocotyledonary buds and shoots does not necessarily insure survival of the injured hypocotyl.

These tests, therefore (as all so-called "physiological" tests with plants which experimentally are injured physically or chemically as well as all so-called "pathological" tests with plants which experimentally are injured infectiously), are made with plants experiencing, and reacting to, an injury—hence, with plants in a pathic state. In the decapitated, untreated hypocotyl this state is not complicated by addition of lanolin or by indoleacetic acid in lanolin. Hence decapitated but untreated hypocotyls were run as controls for the decapitated lanolin controls. The pathology of the decapitated untreated and treated hypocotyl in recovery, and in failure of recovery, will be described in detail in a later paper.

Table 1 reports some of the results of experiments 1–5, which were conducted at various times of the year with decapitated controls, lanolin-treated decapitated controls, and with decapitated hypocotyls treated with various concentrations of indoleacetic acid. The bud counts of these experiments are not given here because of their large number. Table 3 for experiment 7 shows the great variability of such data. Table 1 also reports the number of surviving plants of experiments 1 and 2.

Part of experiment 1 was planned to determine whether lanolin alone and indoleacetic acid in lanolin affect hypocotyledonary bud formation and development in decapitated hypocotyls of flax and to give a clue as to the maximum concentration of indoleacetic acid usable. It was found that one treatment with (*a*) 3% or 1% indoleacetic acid completely inhibited bud formation for the period of bud readings from April 27 to May 16; (*b*) 0.3% or 1:1 (0.116%) indoleacetic acid produced temporary inhibition, the former concentration producing the longer inhibition; (*c*) 1:2 (0.058%) indoleacetic acid produced no inhibition at all. In addition to their effects upon bud formation, concentrations 3%, 1%, and 0.3% produced pathic changes such as temporary blanching of the hypocotyl and excessive swelling directly below the site of application (fig. 1*B*). Furthermore, these pathic effects terminated fatally. Thus in experiments 1 and 2, one month after the last bud count, 99%, 100%, and 70%, respectively, of the plants treated with 3%, 1%, and 0.3% indoleacetic acid had died. These lethal effects are similar to the herbicidal effects

TABLE 1

EFFECTS OF LANOLIN AND INDOLEACETIC ACID UPON BUD FORMATION BY DECAPITATED HYPOCOTYLS OF FLAX AND UPON THEIR SURVIVAL

Experiment no.	History of plants and of treatment	Kind of treatment	Response of hypocotyl	
1.....	Planted 4/15	Decapitated	Buds on 6th day	100% alive
	Up 4/19	" , lanolin	"	100% "
	Treated 4/21	" , 3% indoleacetic acid	No buds on 19th day	99% dead
	Read 4/27, 4/29	" , 1% "	"	100% "
	5/2, 5/4, 5/9, 5/16	" , 0.3% "	No buds on 14th day; buds on 19th day	70% "
		" , 1:1 (0.116%) "	No buds on 6th day; buds on 8th day	100% alive
		" , 1:2 "	Buds on 6th day	100% "
2.....	Planted 4/26	Decapitated	Buds on 6th day	100% alive
	Up 4/29	" , lanolin	"	100% "
	Treated 5/6	" , 1% indoleacetic acid	No buds on 19th day	100% dead
	Read 5/12, 5/14	" , 0.3% "	"	100% "
	5/16, 5/18, 5/20	" , 1:1 "	No buds on 10th day; buds on 12th day	100% alive
	5/23, 7/11	" , 1:2 "	Buds on 6th day	100% "
3.....	Planted 5/20	Decapitated	Buds on 5th day	
	Up 5/24	" , lanolin	"	
	Treated 6/3	" 1:1 indoleacetic acid	No buds on 14th day; buds on 21st day	
	Thereafter daily applications of lanolin and indoleacetic acid	" , 1:2 "	"	"
		" , 1:4 "	"	"
		" , 1:8 "	No buds on 12th day; buds on 14th day	
		" , 1:16 "	No buds on 7th day; buds on 10th day	
		" , 1:32 "	No buds on 5th day; buds on 7th day	
	Read 6/8, 6/10, 6/13, 6/15, 6/24, 7/5	" , 1:64 "	Buds on 5th day	
		" , 1:128 "	No buds on 5th day; buds on 7th day	
4.....	Planted 9/19	Decapitated	Buds on 7th day	
	Up 9/25	" , lanolin	Buds on 7th day	
	Treated 10/4	" , 1:1 indoleacetic acid	No buds on 20th day	
	Thereafter daily applications of lanolin and of indoleacetic acid	" , 1:2 "	"	
		" , 1:4 "	"	
		" , 1:8 "	"	
		" , 1:16 "	"	
	Read 10/11, 10/13, 10/15, 10/17, 10/19, 10/24	" , 1:32 "	Buds on 7th day	
		" , 1:64 "	"	
		" , 1:128 to 1:524,288 inclusive	"	
5.....	Planted 9/29	Decapitated	Buds on 17th day	
	Up 10/4	" , lanolin once daily	"	
	Treated 10/20	" , 1:1 indoleacetic acid once daily	No buds on 26th day	
	Thereafter one-half of each lot daily applications of lanolin and of indoleacetic acid	" , 1:4 " once daily	"	
		" , 1:16 " once daily	"	
	Read 10/27, 10/29, 10/31, 11/2, 11/5	" , 1:64 to 1:262,144, inclusive once daily	Buds on 17th day	
			No buds on 26th day	
			Buds on 17th day	
			"	

FIG. 1.—All plants, of same age from seed planted in quartz sand April 15, had experienced same cultural conditions when severed on May 10, at ground line, for photographing. *A*, *left*, representative control plant showing hypocotyl, cotyledons, short first internode, longer higher internodes; cotyledonary buds beginning to develop into axes. All axes began to flower June 7; buds in leaf axils also had then become grossly detectable. Note that hypocotyl in *A* is longer than those of *B*, *C*, and *D*. Sets *B*, *C*, and *D* are hypocotyls from plants decapitated directly below cotyledonary node on April 21 and recut on April 25, the day of treatment (experiment 1, table 2). *B*, treated with 0.3% indoleacetic acid in lanolin; *C*, treated with 1:1 (0.116%)

(Legend continued on following page)

noted for bean and tomato by LINK, WILCOX, and LINK (4). All untreated controls and all lanolin-treated decapitated plants alive at the time of treatment had survived.

Another part of experiment 1 was designed to determine the effect of a delay of 2 and 4 days in treatment of the decapitated hypocotyls with lanolin and with high concentrations of indoleacetic acid. The plants were decapitated on April 21. One half were not cut again but were treated on the 2d and 4th days after decapitation. The other half were divided into three lots. The first of these was treated immediately after decapitation; the second, 2 days, and the third, 4 days, after decapitation (fig. 1). Presence or absence of buds is shown in table 2 for the days on which readings were made.

The results indicate that concentrations of 3%, 1%, 0.3%, and 0.116% indoleacetic acid were more effective in inhibiting or retarding bud formation if applied on the day of decapitation than on later days. The acid seemed to be equally effective whether applied to a new cut or to the old cut surface.

Concentrations of 3% and 1% indoleacetic acid applied on the day of decapitation completely inhibited bud formation until the plants died. A concentration of 0.3% applied on that day delayed it for 18 days, but the plants died. Applied 2 and 4 days after decapitation, 3% and 1% indoleacetic acid delayed bud formation for 13 days. Because of the findings of this test, treatment in all subsequent experiments was applied on the day of decapitation.

Experiment 2 was a repetition of part of experiment 1 with elimination of the treatment with 3% indoleacetic acid. The results were essentially those obtained in experiment 1 as to effects on budding and on death of the hypocotyl.

In experiment 3 the hypocotyls were treated daily with lanolin and with indoleacetic acid, and higher dilutions of indoleacetic acid were used—1:1 (0.116%) to 1:128 (0.0009%). The plants were grown during the period of maximum sunshine (June 3–July 5). In addition to verifying the results of experiments 1 and 2, the data of this experiment indicate that repeated applications of indoleacetic acid in the higher dilutions were more effective in producing inhibition of bud formation than a single application of the same concentration. Thus concentration 1:32 (0.0036%) indoleacetic acid applied daily produced a 10-day retardation of grossly detectable bud formation, whereas a single application of concentration 1:2 (0.058%) in experiments 1 and 2 did not produce any retardation or inhibition.

Experiment 4 was a duplication of ex-

(Continued from preceding page)

indoleacetic acid in lanolin; *D*, treated with lanolin paste and used as controls. Note substitute axes in *D*; when more than one developed, one became dominant. It flowered a few days later than axes of uncut plant but was shorter and more slender. Also note additional buds in center plant. Plants treated with lanolin immediately and 2 days after decapitation resembled these plants. In other experiments, concentrations of 0.3% and 1:1 indoleacetic acid, when applied immediately after decapitation, completely and permanently inhibited initiation and development of bud primordia and bud and shoot development in upper and lower halves of hypocotyl. Concentration 1:128 (0.0009%) was highest dilution which significantly retarded bud initiation and development (experiment 1, table 3 and figs. 2, 3). Shoots of set *C* are shorter than those of set *D*. Note club-shaped tumefaction of hypototyl in set *B*; complete absence of buds in specimen to left and increasing numbers of buds scattered along entire length of hypocotyl, as well as small shoots in other specimens. These buds did not appear until 14 days after treatment, a delay of 12 days, and then appeared only in 30% of plants. These hypocotyls died prematurely, as did those treated with 1:1 indoleacetic acid. Note in table 2 that 0.3% indoleacetic acid produced complete and permanent inhibition in hypocotyls treated on date of decapitation.

periment 3 and, in addition, tested dilutions of indoleacetic acid up to and including 1:524,288 (0.0000012%). The light conditions were not so favorable as in experiment 3, but the results were essentially the same.

In experiment 5 the effects of one and of daily treatments were tested concurrently. Light conditions were not so favorable as in experiment 4, but the findings corroborate those of experiments 3 and 4. In the higher concentrations—1:1 (0.116%) and 1:4 (0.029%) indoleacetic acid—the single treatment and the daily treatment produced the same effects. Concentration 1:16 (0.00725%) indoleacetic acid showed a differential effect, the daily treatments delaying bud formation longer than the single treatment.

Perusal of the results of experiments 1–5 indicates that the effectiveness of a particular concentration within the maximum range of effectiveness varied slightly in the different experiments. The time of bud appearance in the controls and lanolin-treated plants ranged from 5 (3d experiment [May 20–July 5]) to 17 days (5th experiment [September 29–November 5]). Plants treated with 1:1 (0.116%) indoleacetic acid did not produce buds until the 8th day in experiment 1 (April 27–May 16) and not until the 12th day in experiment 2 (May 12–July 11). Buds appeared by the 21st day in experiment 3 (June 8–July 5) in plants treated daily with concentration 1:4 (0.029%), whereas in experiment 5 the same treatment held buds back beyond the 26th day. In this test, concentration 1:16 (0.00725%) held back buds past the 26th day, whereas in experiment 3 this

TABLE 2

PRESENCE OR ABSENCE OF BUDS FOLLOWING DECAPITATION AND DEFERRED TREATMENT AND FOLLOWING DECAPITATION WITH REPEATED CUTTING AND IMMEDIATE TREATMENT

Date of treatment	Date of reading	Kind of treatment											
		Decapitation 4/21; new cut on day of treatment						Decapitation cut of 4/21					
		Control	Lanolin	Indoleacetic acid				Control	Lanolin	Indoleacetic acid			
				3%	1%	0.3%	1:1			3%	1%	0.3%	1:1
4/21	4/27	+	+	o	o	o	o	.....	.....	.....	.....	.....	.....
	4/29	+	+	o	o	o	+	.....	.....	.....	.....	.....	.....
	5/2	+	+	o	o	o	+	.....	.....	.....	.....	.....	.....
	5/4	+	+	o	o	o	+	.....	.....	.....	.....	.....	.....
	5/9	+	+	o	o	+	+	.....	.....	.....	.....	.....	.....
4/23	4/27	+	+	o	o	o	o	+	+	o	o	o	o
	4/29	+	+	o	o	o	+	+	+	o	o	o	+
	5/2	+	+	o	o	+	+	+	+	o	o	o	+
	5/4	+	+	o	o	+	+	+	+	o	o	+	+
	5/9	+	+	+	+	+	+	+	+	+	+	+	+
4/25	4/27	+	+	o	o	o	+	+	+	o	o	o	+
	4/29	+	+	o	o	o	+	+	+	o	o	o	+
	5/2	+	+	o	o	+	+	+	+	o	o	+	+
	5/4	+	+	o	o	+	+	+	+	o	o	+	+
	5/9	+	+	+	+	+	+	+	+	+	+	+	+

concentration retarded them only 7–9 days. These results probably indicate that the metabolic status of the plant is a factor in determining the effectiveness of various concentrations of indoleacetic acid (**2, 3, 6**) in affecting bud formation and development.

Some data of experiments 1–5 and of other experiments indicated at times that lanolin possibly affects the number of buds formed. They also indicated that treatments immediately after decapitation and after a lapse of 1–6 hours, respectively, produced differences in the number of buds formed and also that a single treatment either immediately after decapitation or after some hours on the same day produced different results than did a series of treatments on successive days.

The data of experiments 1–5 also had indicated the possibility that concentrations of indoleacetic acid ranging from 1:256 (0.00045%) to 1:524,288 (0.0000012%) at times increased the number of buds formed and developed by the treated hypocotyl. To determine whether the differences noted in the average of buds formed were statistically significant, experiment 6 was set up. It included the following eleven test lots, each comprising five pots with twenty plants each: (1) cut, untreated control; (2) lanolin, one treatment applied immediately after decapitation; (3) lanolin, one treatment applied 6 hours after decapitation; (4) lanolin, first treatment applied immediately after decapitation, thereafter daily; (5) lanolin, first treatment applied 6 hours after decapitation, thereafter daily; (6) indoleacetic acid 1:4096 (0.000028%), one application immediately after decapitation; (7) indoleacetic acid 1:4096, one application 6 hours after decapitation; (8) indoleacetic acid 1:4096, first application immediately after decapitation, thereafter daily; (9) indoleacetic acid 1:4096, first application 6 hours after decapitation, thereafter daily; (10) indoleacetic acid 1:8192 (0.000014%), one application immediately after decapitation; and (11) indoleacetic acid 1:16,384 (0.000007%), one application immediately after decapitation. This experiment was conducted from March 7 to April 10. Readings were made on the 3d, 5th, 9th, and 15th days after buds first had begun to appear. Readings of the number of buds counted on the 9th day were treated statistically. No statistical significance was found for the differences noted between test lots 1 and 2–11, inclusive; 2 and 3; 4 and 5; 6 and 7; 8 and 9; 6 and 8; and 7 and 9. This indicates that the differences noted were due to individual variation in the flax plants tested and that the flax plant and the methods used are not suitable to determine whether indoleacetic acid in certain concentrations stimulates bud formation and development in the decapitated hypocotyl.

The preliminary experiments and experiments 1–5 also had indicated that certain higher concentrations of indoleacetic acid—1:1 (0.116%) to 1:256 (0.00045%)—which are not high enough completely to inhibit bud formation and development, are adequate to reduce the number of buds formed per plant. The threshold seems to vary with the metabolic state of the plant.

Experiment 7 was set up so that statistically usable data might be obtained to determine the validity of these indications. The experiment included the five following test lots, each comprising six pots of twenty plants each: (1) cut, untreated control; (2) lanolin, one treatment; (3) indoleacetic acid 1:1 (0.116%), one treatment; (4) indoleacetic acid 1:128 (0.0009%), one treatment; and

(5) indoleacetic acid 1:4096 (0.000028%), one treatment. Lanolin and indoleacetic acid were applied immediately after decapitation. Readings were begun 7 days after treatment and were made again on the 9th, 11th, 15th, and 21st days. The data obtained on the 15th day were treated statistically. Table 3 records the data of all readings in terms of percentage of plants budded and of average number of buds per plant. Figure 2 shows the percentage of plants budded and figure 3 the average number of buds per plant for all readings.

Calculation of SE and t showed that the differences between the cut, untreated control (1) and the lanolin-treated decapitated hypocotyl (2) are not statisti-

TABLE 3

VARIABILITY IN RATE OF BUD FORMATION BY HYPOCOTYLS OF FLAX DECAPITATED AND TREATED ON FEBRUARY 22

Date of reading	Pot no.	Decapitated		Lanolin		Indoleacetic acid 1:4096		Indoleacetic acid 1:128		Indoleacetic acid 1:1	
		Plants budded (%)	Average buds per plant	Plants budded (%)	Average buds per plant	Plants budded (%)	Average buds per plant	Plants budded (%)	Average buds per plant	Plants budded (%)	Average buds per plant
3/1.....	1	23.5	0.41	8.6	0.21	16.6	0.25	0	0	0	0
	2	18.1	0.27	26.3	0.36	33.3	0.38	0	0	0	0
	3	15.7	0.21	31.2	0.62	4.76	0.09	0	0	0	0
	4	5.5	0.05	4.34	0.04	11.1	0.11	0	0	0	0
	5	20.0	0.20	4.76	0.14	16.6	0.16	0	0	0	0
	6	21.7	0.26	10.00	0.10	13.6	0.13	0	0	0	0
	1–6	17.6	0.23	13.1	0.22	16.1	0.19	0	0	0	0
3/3.....	1	47.0	1.41	73.9	2.21	66.6	1.79	31.5	0.42	0	0
	2	72.7	1.95	89.4	3.21	66.6	2.28	86.1	0.86	0	0
	3	36.8	1.05	81.2	2.37	47.6	1.14	5.0	0.05	0	0
	4	44.4	1.00	82.6	2.65	66.6	2.16	0	0	0	0
	5	45.0	0.95	52.3	1.04	61.1	2.05	0	0	0	0
	6	47.8	1.00	65.0	1.44	59.0	1.77	0	0	0	0
	1–6	49.5	1.23	73.7	2.13	61.2	1.85	6.6	0.08	0	0
3/5.....	1	94.1	3.41	91.3	3.91	81.5	3.91	68.4	2.78	0	0
	2	100.0	3.77	94.7	5.00	100.0	3.57	100.0	3.34	0	0
	3	84.2	2.84	93.7	5.62	95.2	2.90	55.0	1.20	0	0
	4	83.3	2.33	91.3	4.56	100.0	3.22	79.1	2.00	0	0
	5	89.4	3.47	90.4	2.66	88.8	3.83	52.1	1.30	0	0
	6	91.3	3.04	90.0	3.35	86.3	3.86	40.7	1.22	0	0
	1–6	91.5	3.16	91.7	4.12	92.7	3.56	65.4	1.94	0	0
3/9.....	1	100.0	9.23	100.0	7.81	95.8	7.08	89.4	3.52	0	0
	2	100.0	9.40	100.0	8.36	100.0	7.47	100.0	5.60	0	0
	3	100.0	9.26	100.0	8.62	100.0	4.76	95.0	2.45	0	0
	4	100.0	8.88	100.0	9.47	94.7	6.05	95.8	3.54	0	0
	5	100.0	9.00	100.0	6.19	100.0	6.27	86.3	2.72	0	0
	6	100.0	7.73	100.0	6.95	95.4	7.45	85.1	3.14	0	0
	1–6	100.0	8.88	100.0	7.90	97.6	6.55	91.8	3.59	0	0
3/15.....	1	100.0	10.47	100.0	8.13	100.0	8.25	89.4	3.88	0	0
	2	100.0	10.09	100.0	9.00	100.0	8.70	100.0	6.08	0	0
	3	100.0	10.15	100.0	9.31	100.0	6.29	100.0	3.10	0	0
	4	100.0	10.11	100.0	10.26	100.0	7.50	95.8	4.58	0	0
	5	100.0	9.80	100.0	7.04	100.0	7.77	86.3	2.95	0	0
	6	100.0	9.50	100.0	7.60	100.0	9.18	92.5	3.70	0	0
	1–6	100.0	10.00	100.0	8.55	100.0	7.97	94.47	4.08	0	0

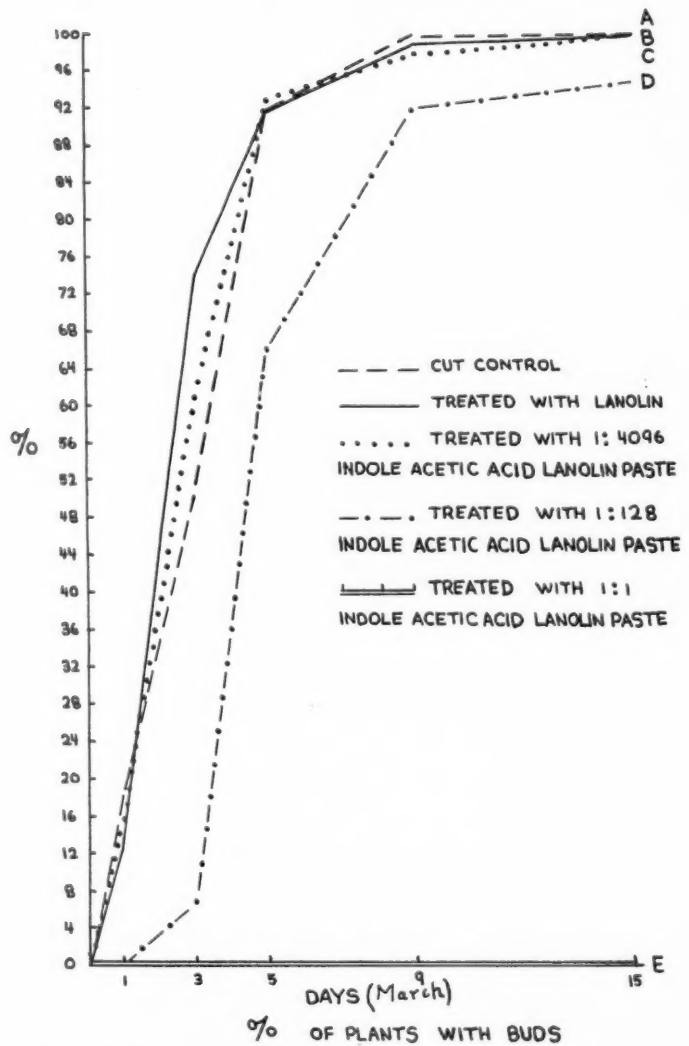

FIG. 2.—Percentage of plants with buds based on counts made March 1, 3, 5, 9, and 15, and tabulated in table 3. *A*, cut, untreated control plants; *B*, decapitated lanolin-treated control plant; *C*, decapitated plants, treated with 1:4096 (0.000028%) indoleacetic acid–lanolin; *D*, decapitated plants, treated with 1:128 (0.0009%) indoleacetic acid–lanolin; *E*, decapitated plants, treated with 1:1 (0.116%) indoleacetic acid–lanolin.

cally significant; that the differences between the lanolin-treated hypocotyls (2) and those treated with indoleacetic acid 1:128 (0.0009%) (4) are highly significant.

The findings of this experiment corroborate those of experiment 6 to the effect that lanolin treatment produces no significant difference in the rate and amount of budding in the treated plant as compared with the cut, untreated plant; that indoleacetic acid in greater dilution than 1:128 (0.0009%) produces no significant changes in the number of buds formed; and that indoleacetic acid 1:1 (0.116%) may produce complete inhibition of bud formation.

When hypocotyls treated with various

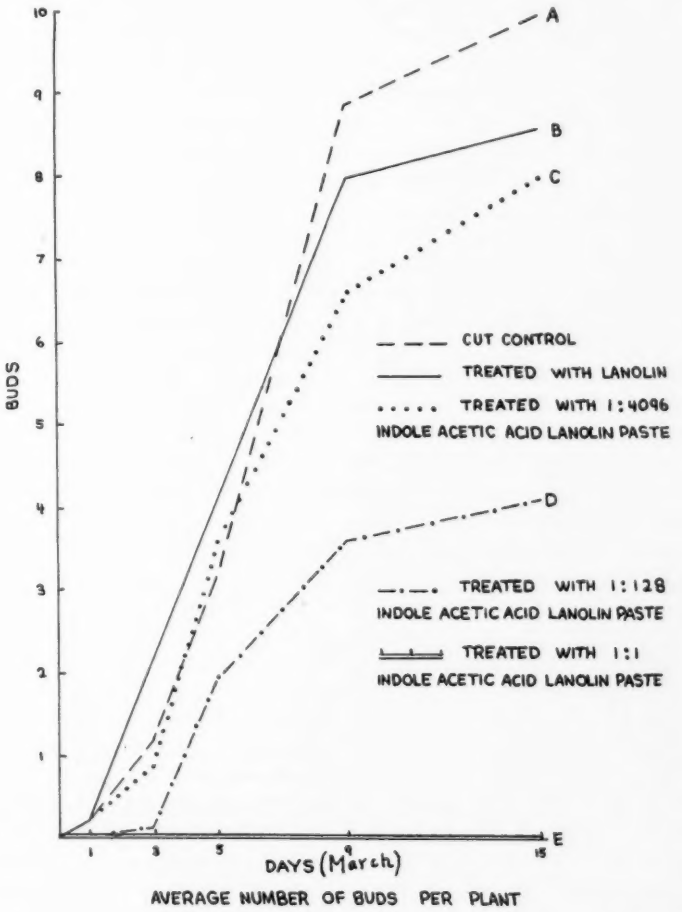

FIG. 3.—Average number of grossly detectable buds per plant, based on counts made March 1, 3, 5, 9, and 15 and tabulated in table 3. *A*, *B*, *C*, *D*, and *E* represent same plants as in fig. 2. Differences between *A* and *B*, *A* and *C*, and *B* and *C* on March 9 are not statistically significant; differences between *A* and *D*, *A* and *E*, *B* and *D*, and *B* and *E* are.

concentrations of indoleacetic acid were examined histologically, it was found that, in those instances in which indoleacetic acid produces complete inhibition of grossly visible buds, the initiation of bud primordia in the upper half of the hypocotyl is completely inhibited and the development of the primordia present in its lower half also is completely inhibited. In those instances in which formation and development of buds are temporarily inhibited and retarded by indoleacetic acid, these effects are due to temporary inhibition in the initiation of bud primordia in the upper half of the treated hypocotyl and to temporary inhibition of development of the primordia present in its lower half.

Discussion

It is evident from the results of the experiments that indoleacetic acid could serve in the intact flax plant as an agent in the inhibition of (*a*) bud primordium initiation; (*b*) development of the primordium into a bud; and (*c*) development of the buds into shoots. Tests to determine whether auxins are present in the flax plant and whether they are so distributed as to serve in inhibition of hypocotyledonary bud primordia in the intact plant are the subject of researches to be reported in other papers.

VAN OVERBEEK (7) reported that 0.1% indoleacetic acid in lanolin applied to the decapitated pea plant completely inhibited development of the lateral buds. In some of our experiments 0.116% indoleacetic acid completely inhibited formation of hypocotyledonary bud primordia and of their development into buds and, of these, into shoots. Concentrations higher than this (i.e., 0.3%–3%) also inhibited these events, but they also led to premature death of the treated organ after stimulating it to excessive tumor formation beneath the treated wound.

Our failure to establish whether any of the concentrations of indoleacetic acid tested incites or hastens bud primordia formation and development of these into buds may indicate that, in addition to auxins, factors other than indoleacetic acid—and not controlled in our tests—also play a role in bud primordium initiation and in bud development.

The results of our experiments show that indoleacetic acid and presumably the auxins of the flax plant so affect the epidermal cells of the hypocotyl that they do not experience the transverse cell divisions characteristic of bud primordium initiation or the subsequent divisions and other cell processes characteristic of development of the primordium into a bud and of development of the bud into a shoot. They do not, however, indicate how these effects are brought about. Presumably they could be affected directly or indirectly.

It is evident that the effects are quite definite or specific so far as cell division is concerned; while cell divisions characteristic of bud primordium initiation are inhibited, cell divisions involved in formation or development of wound tissue are not inhibited.

The fact that repeated applications of a given concentration of indoleacetic acid are more effective than one application in inhibiting bud formation and development indicates that attainment and maintenance of a certain threshold value is essential for continued inhibition of bud initiation and development and that the indoleacetic acid applied is used up, or inactivated. The fact that a certain concentration, unless renewed, inhibits bud formation for only a limited time points to the same conclusion.

Delay for more than a day in treatment with a concentration which is inhibitory if applied immediately retards but does not completely inhibit bud formation. This suggests that this given concentration of indoleacetic acid more readily inhibits the epidermal cell division involved in bud primordium initiation than it does the processes involved in development of the primordium into a bud and of the bud into a shoot.

There are many studies dealing with the inhibitory effects of auxins upon the development of lateral buds, and several theories about the action of auxin in producing this effect, which have been critically evaluated by VAN OVERBEEK (7). We are aware, however, of only one report on the effects of auxins upon initiation of buds which are not mediated by callus tissue. BEAL (1) reported that *Lilium harrisii*, when decapitated and treated on the cut surface with 3% indoleacetic acid in lanolin, initiated buds in the axils of leaves which normally do not bear buds. He described the course of events as follows:

> In *L. harrisii* the region of visible response to the treatment is limited largely to the cells of the epidermis and outer cortex in the immediate vicinity of the leaf axil. The epidermal cells of the stem immediately above the axil elongate radially, while the outer cortical cells centripetal to, as well as slightly below, them enlarge and in the course of about 5 days begin dividing. This marks the initiation of the region at which buds will later develop.

At the time we undertook the experiments reported here, we entertained the hypothesis that a range of concentration might be found which would stimulate the epidermis of the decapitated flax hypocotyl to initiate buds. For a time, as pointed out above (5), it seemed as though we had found, in certain experiments, concentrations of indoleacetic acid which did produce this effect. Had this finding been substantiated, we should be dealing with a situation in which an auxin (indoleacetic acid) induces cell division without first inducing cell enlargement. Possibly no concentration of indoleacetic acid plays a role in inciting this train of events. Unfortunately, either the great variation in capacity of individual hypocotyls to initiate buds, or the essentially uncontrolled metabolic status of our plants, or both, made it impossible to obtain statistically significant data bearing upon the question of whether indoleacetic acid stimulates epidermal cells to divide transversely and thus to begin the initiation of bud primordia. The findings show clearly, however, that a range of concentration of indoleacetic acid exists which inhibits and retards the cell divisions which initiate buds in the decapitated hypocotyl of flax.

We are not concerned here with the interesting questions as to whether this effect of auxin is brought about directly or indirectly and what the mechanism is whereby it is brought about. These questions will be discussed in later papers.

The findings indicate that an auxinic mechanism may be a method whereby initiation and subsequent development of hypocotyledonary bud primordia are controlled in the intact flax plant. Studies of the auxinic situation in the intact flax plant, and in isolated members of it, in its possible relation to bud inhibition will be reported on in later papers.

The finding that for a given concentration of indoleacetic acid, in any particular experiment, repeated applications are more effective than a single application may indicate that the auxin applied is used up or bound in some way and that

repeated applications are essential for maintenance of the threshold concentrations necessary to inhibit bud initiation and development. These thresholds seem to vary with the metabolic or nutritional status of the plant prior to and following decapitation and treatment.

Summary

1. The decapitated hypocotyl of flax, with its initial injury and the resultant injury effects, is in a pathic state during the course of the experiments reported here. The metabolic status of the hypocotyl at the moment of infliction of the injury, and the external conditions to which it is exposed after its mutilation, determine to what extent the injury effects terminate in recovery and shoot regeneration, or in death.

2. Single or repeated applications of lanolin alone to the wound of the decapitated flax hypocotyl produced no detectable effects upon survival of the hypocotyl or upon its initiation and development of hypocotyledonary buds.

3. Applications of 3% and of 1% indoleacetic acid in lanolin to the decapitation wound completely and permanently inhibited bud primordium development in the lower half, and bud primordium initiation in the upper half, of the hypocotyl. The treated plants died earlier than the untreated cut controls, as reported earlier for bean and tomato.

4. Application of 0.3% indoleacetic acid in lanolin retarded these bud events for a long time. It also led to the premature death of most of the treated hypocotyls. Application of 1:1 (0.116%) indoleacetic acid in lanolin led to slight retardation or to complete inhibition of these bud events, depending upon the number of treatments and upon the metabolic status of the tested plants.

5. The lowest concentration of indoleacetic acid noted to retard bud primordium initiation and development was 1:128 (0.0009%). One treatment with this concentration in February was more effective than repeated treatments in May.

6. While one treatment with 1:16 (0.007%) concentration of indoleacetic acid retarded grossly detectable bud appearance for 17 days, repeated treatment suppressed it completely for 26 days.

7. In the higher concentrations, 1:4 (0.029%) and above, no difference was noted in the effect of single as compared with repeated treatments.

8. Under the conditions of the experiment no concentration was found which stimulated bud primordium initiation and development.

9. The findings show that indoleacetic acid in lanolin may retard or completely inhibit the transverse divisions of epidermal cells of the hypocotyl which are the first detectable beginning of hypocotyledonary bud primordium initiation in flax.

10. The greater efficiency of repeated applications than of a single application of the same concentration of indoleacetic acid in inhibiting or retarding these events may indicate that the former treatment maintains the threshold necessary for inhibition by replacing auxin used up or bound in the hypocotyl.

DEPARTMENT OF BOTANY
UNIVERSITY OF CHICAGO
AND
MOUNT SINAI HOSPITAL
CHICAGO, ILLINOIS

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MEGASPOROGENESIS AND DEVELOPMENT OF THE EMBRYO SAC OF ALLIUM CERNUUM

JOHN B. MURPHY

Introduction

During recent years much previous work on the Liliaceae has been questioned. Reinvestigation has, in some cases, effected grave changes in the pattern of the life-story of members of the family. The *Lilium* type of embryo sac, established by TREUB and MELLINK (**36**) as a result of their study of *Lilium bulbiferum*, has lately been eliminated because of the subsequent reinvestigation by BAMBACIONI (**1, 2, 3**). Investigators such as GUIGNARD (**11**), SARGANT (**31**), COULTER (**7**), and MOTTIER (**25**) had observed pecularities in the development of the *Lilium* megagametophyte, but considered these to be abnormalities and failed to investigate them any further. BAMBACIONI established that the mature embryo sac of *L. bulbiferum* followed the pattern of the *Fritillaria* type as first described in her report on *F. persica* and *L. candidum*. COOPER (**5, 6**) suggested that the *Fritillaria* type is probably characteristic of the whole genus *Lilium*.

The present investigation was undertaken to make a comparative study of megasporogenesis and embryo-sac development in a genus related to *Lilium* with special attention to the type of embryo sac and its possible variation from the traditional type. An uninvestigated species, *Allium cernuum* Roth, was considered a favorable species for such study.

Material and methods

Ovaries were collected at intervals over a period extending from June through August in the years 1943 and 1944. They were prepared for sectioning by the usual paraffin technique. Some were stained with safranin and fast green; others were stained with safranin, gentian violet, and orange G in clove oil. Sections cut at 8 or 10 μ were found most suitable for study.

Observations

Development of the megasporophylls

Three carpel primordia appear as crescent-shaped mounds in the flattened area of the flower summit inside the inner whorl of stamens and alternate with

them. The bases of the primordia grow more rapidly than the apices, thus causing the megasporophylls to be leaflike in appearance, with a broad base and a bluntly pointed apex. The subsequent growth of the carpels results in syncarpy by an inward arching of the three segments until eventually the inturned margins come in direct contact with each other; at the point of contact the carpels fuse, forming the style primordium. The primordium of the compound pistil grows upward, resulting in formation of a simple, persistent, threadlike style arising from the tip of the ovary.

The basal portions of the carpels constitute the ovary. At about the time that the style initial begins to elongate, the ovule primordia differentiate from the inner ridges of the carpellary margins in the ovarian portion, thus showing axillary placentation.

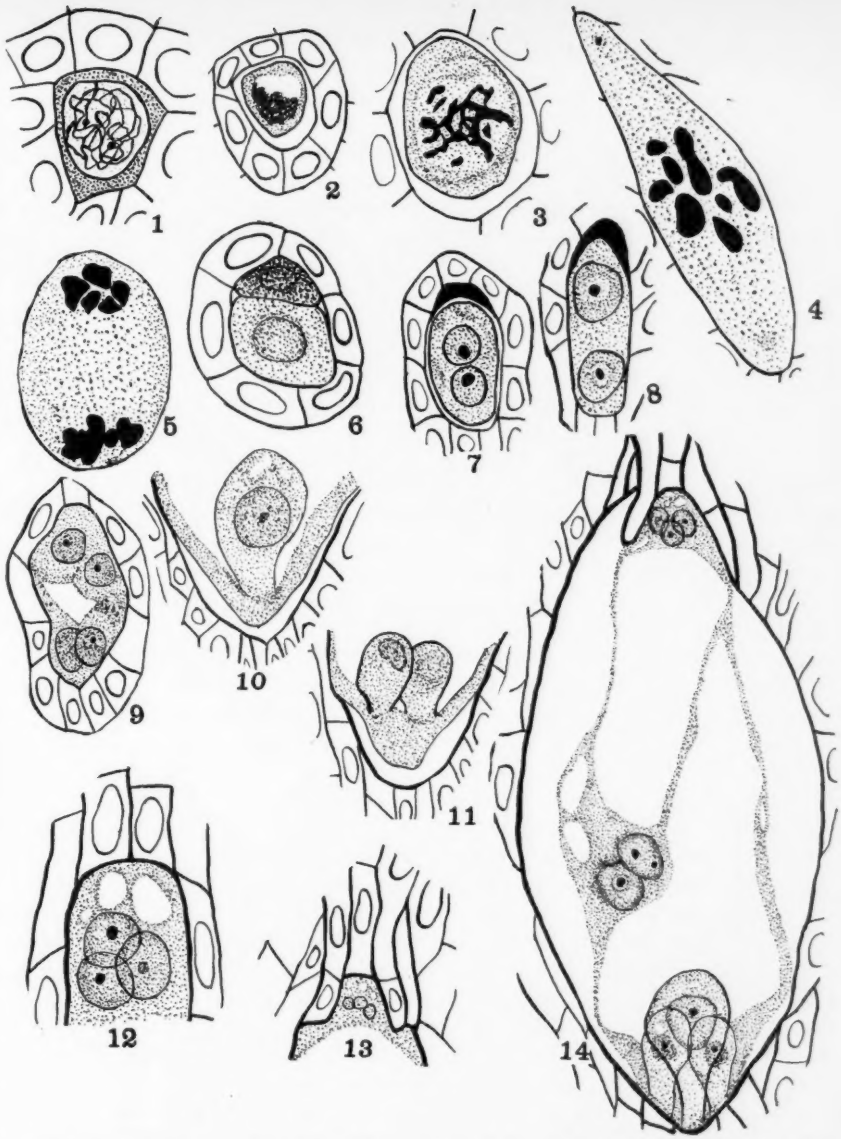
Development of the megasporangium

The ovule initial appears as a dome-shaped mass of cells which arises from the placenta at the base of the carpel. Periclinal divisions of cells of the hypodermal layer of the sporophyll result in the formation of a mass of nucellar tissue. The overlying epidermal cells divide only anticlinally, thus compensating for the enlargement of the nucellus and, at the same time, forming a layer of epidermal tissue. The nucellus develops rapidly, and, before the style is very far developed, a single hypodermal archesporial cell is differentiated. Shortly after the archesporium is formed, the inner integument arises by periclinal cell division as an annular fold of the epidermis. The inner integument develops rapidly, and, by the time the archesporium is in synapsis, the outer integument has also appeared and is considerably elongated. The integuments grow with greater rapidity than the nucellar tissue and completely envelop the ovule before meiosis is completed. The inner integument varies from three to five rows of cells in thickness at maturity and is slightly shorter than the outer integument, which is seven to nine cell rows thick. Both integuments are tightly pressed together, but at the time of pollination the cells of the integuments contract, resulting in a micropylar opening. After the entrance of the pollen tube the integuments again become tightly pressed together.

During early stages of the megagametophyte the ovule undergoes a complete inversion, resulting in its anatropous orientation. In the process of anthesis there is a rapid growth of the chalazal tissue, and the funiculus becomes sharply curved just below the chalaza; as a result, the integuments are inverted and lie alongside the stalk of the funiculus, with the micropyle directed toward the placenta. The integuments reach maturity shortly before anthesis is completed.

Development of the megagametophyte

The archesporium differentiates about 3 or 4 days before the flowers emerge from the bud, which is about the time that the floral envelope starts turning to its white or rose color. The archesporial cell (fig. 1) forms before the initials of the inner integument appear and functions directly as the megaspore mother cell. The megaspore mother cell undergoes the first reduction division, resulting in two homomorphic dyads (fig. 6). Phases of this division are seen in figures 1–6. Judging by the few sections found in the dyad stage, it may be concluded that this stage is very brief. After the first meiotic division the micropylar dyad begins to



FIGS. 1–14.—Camera lucida drawings. In figures 1–9 the micropylar end of megagametophyte is toward top of page. Fig. 1, cross-section of megaspore mother cell, early prophase (950×). Fig. 2, megaspore mother cell, synizesis (440×). Figs. 3–5, late prophase, metaphase, and anaphase of heterotypic division (950×). Fig. 6, late stage of dyads. Micropylar dyad beginning to disintegrate (950×). Fig. 7, two-nucleate megagametophyte. Micropylar dyad nearly disintegrated (440×). Fig. 8, older two-nucleate stage, nuclei migrating to opposite ends of sac (440×). Fig. 9, reconstruction from two sections, four-nucleate stage of megagametophyte. Micropylar megaspore completely disintegrated (440×). Fig. 10, transverse section of egg cell (950×). Fig. 11, orientation of two synergid cells (950×). Fig. 12, cross-section through antipodal cells and heavily stained nucellar cell walls (950×). Fig. 13, disintegrating antipodal cells and heavily stained chalazal nucellar cell walls (950×). Fig. 14, reconstruction from three sections, mature eight-nucleate megagametophyte (950×).

disorganize, apparently without any further nuclear division (figs. 6, 7, 8). The chalazal dyad enlarges, forms a new achromatic figure, and undergoes a second reduction division, resulting in two homomorphic megaspore nuclei which migrate to the opposite ends of the cell (figs. 7, 8). The cell does not form a cross-wall. The binucleate megaspore stage lasts relatively long. Both of these megaspores take part in the formation of the embryo sac, thus placing it in the bisporic "*Allium* type." The disintegrating dyad is still visible at this stage (fig. 8), although the residue is rapidly being digested and assimilated by the surrounding cells.

After the second meiotic division small vacuoles begin to appear, and the megagametophyte enlarges to almost twice its original size (fig. 8). Both megaspore nuclei now divide again, giving rise to the four-nucleate stage (fig. 9), two nuclei being oriented at each end of the cell. Following this division, the megagametophyte becomes increasingly vacuolated, almost doubling the dimensions of the two-nucleate stage (fig. 9). The central portion of the sac is practically one large vacuole. The nuclei at each end of the sac are side by side in the same plane though not touching each other. The micropylar nuclei, however, are not in the same plane as the chalazal nuclei. Figure 9 is a composite of the two planes. The disintegrating dyad has been completely absorbed by the time this stage is reached. The four-nucleate stage is apparently of short duration Each of these four nuclei undergoes a second mitosis, resulting in the formation of an eight-nucleate megagametophyte. For a short while it continues to increase in size, primarily as the result of vacuolization (fig. 14).

One nucleus migrates from each end of the sac toward the central peripheral region, where they approximate each other (fig. 14). These polar nuclei are about equal in size and chromatic density. The three nuclei remaining in the micropylar end organize into a group of three cells (fig. 14). The egg cell is much larger than the synergids and is pear shaped. The synergid cells (figs. 11, 14) are about equal in size and density and lie behind the egg. One of the synergids seems to disintegrate shortly before fertilization.

The antipodals organize into cells and form a rather loose mass in the chalazal region (fig. 14). In some cases they are about equal in size and density and may persist throughout the maturation of the megagametophyte (fig. 12), but more frequently they are very small (fig. 13) or may even be absent.

After the orientation of the nuclei the megagametophyte grows rapidly to its mature size (fig. 14). Cytoplasmic vacuolation causes most of the cytoplasm to be massed at the micropylar end around the egg and synergids. A smaller amount of dense cytoplasm is gathered around the polar nuclei, and a still smaller amount lies at the chalazal end surrounding the antipodals (fig. 14). Small vacuoles are often found between the antipodals and the end of the sac (figs. 12, 13).

The mature megagametophytes examined were about 198 μ in length and 98 μ in width, being narrow and more pointed at the chalazal end of the sac. The antipodal region of the protoplasm is wedged as a small pocket into the surrounding tissue (fig. 13). The megagametophyte is mature about 6–12 hours after the opening of the flower, and fertilization occurs within 3 days after the flower bud opens.

CHROMOSOMES.—The number of chromosomes in the megagametophyte was determined as eight (fig. 4).

NUCELLUS

The nucellar cells adjacent to the megagametophyte are somewhat different from the others. As the archesporium differentiates, the inner nucellar cells divide periclinally, forming a distinct layer, the walls of which thicken as the megagametophyte develops. As the megagametophyte reaches maturity and its nuclei become oriented, the walls of the surrounding cells attain their greatest thickness at the chalazal and micropylar ends. The cells immediately surrounding the chalazal end of the megagametophyte are distinctly larger, thicker walled, and differently shaped than the other nucellar cells. They are long, blunt at one end, and taper toward the megagametophyte, which seems to be wedged into these cells (figs. 12, 13, 14). The antipodals are generally oriented in such a way as to be harbored under the pointed ends of these projecting cells (figs. 13, 14). The inner walls of the nucellar cells surrounding the micropylar end of the megagametophyte are also unusually thick and of characteristic shape (figs. 10, 14). The walls of the nucellar cells along the rest of the megagametophyte are also slightly thicker than usual (fig. 14). The wall thickening of the nucellar cells surrounding the megamametophyte is noticeably diminished after fertilization.

Discussion

Although a number of variations have been reported in the development of the megagametophyte of several species of *Allium*, this investigation shows that *A. cernuum* follows the characteristic bisporic *Allium* type (formerly called the "Scilla type") as first described by STRASGURGER (**34**) for *A. fistulosum*. This investigation shows, moreover, that the chalazal dyad undergoes three successive divisions to form the eight-nucleate megagametophyte, whereas the micropylar dyad disintegrates without further division. SCHURHOFF (**33**), MODILEWSKI (**23, 24**), and JONES and EMSWELLER (**16**) made similar observations in other species of *Allium*. WEBER (**37**) noted that the megagametophyte frequently originated from the micropylar dyad in many of the species that she investigated. MESSERI (**22**) observed that a large number of the megagametophytes belonged to the monomegasporal type. PORTER (**28**), in a recent description of *A. mutabile* Michx., stated that the chalazal dyad undergoes another division on forming two megaspores of which the chalazal one functions. This would place the megagametophyte of *A. mutabile* in the normal monosporic type. In the present investigation particular attention was focused on *A. cernuum* for evidence of a similar occurence, but no such observation was made. The evidence of PORTER and of MESSERI seems incomplete and demands a thorough reinvestigation.

Many megagametophytes included under the *Allium* type are reported to have less than the usual eight nuclei. MESSERI considered it sufficiently common in the several species she studied to establish a "seven-nucleate" *Allium* type. The limitation occurs in the chalazal end of the megagametophyte after the four-nucleate stage. No such observation was made in this investigation, and in every case the megagametophyte contained the typical eight nuclei. The "egglike" antipodal cells observed by MESSERI in *A. subhirsutum* and *A.*

schoenoprasum, and by MODILEWSKI in *A. nigrum*, were not found in *A. cernuum*. On the contrary, this investigation confirms the report of ELMORE (10) that the mature gametophyte shows a more or less marked reduction of the antipodal apparatus and, in many cases, even a complete loss of antipodals.

There are two synergids in *A. cernuum*. They are about equal in size and density in contrast with the one large and one small synergid observed by JONES and EMSWELLER in *A. cepa*, or with only the one synergid observed by PORTER in *A. mutabile* Michx. A "filamentous apparatus" in the synergids, as reported by MESSERI, was not observed in *A. cernuum*, nor was one of the synergids hypertrophied, as found in *A. cepa* (16). One of the synergids, however, showed rapid disintegration just prior to fertilization. The statement of PORTER that the egg and synergid are sister cells and that the polar nuclei are also sister cells finds no confirmation in this study. However, the thick-walled cells of the inner nucellus, as reported in *A. mutabile* Michx. (28), are characteristic of *A. cernuum*.

The author was interested in the theory that a species of *Allium* might possibly belong to the *Fritillaria* type as described by BAMBACIONI and by COOPER for many species of *Lilium*. *Allium cernuum* does not, however, deviate from the usual *Allium* type.

A comparative study of megagametophyte development in other genera of the *Allium* type offers an interesting variation. HEATLY (13), in *Trillium cernuum*, MCALLISTER (20), in *Streptopus roseus*, and MCKENNEY (21), in *Scilla hyacinthoides* var. *caerules* and *S. campanulata* found that the embryo sac develops from the micropylar dyad. SVENSSON (35) observed that the micropylar dyad starts to divide in *Lycopsis arvensis* but degenerates before the division is completed. In *S. nonscripta*, as described by HOARE (14), the micropylar dyad functions and the chalazal dyad disintegrates, although it first undergoes a division forming an "antigone" consisting of four nuclei. The Alismaceae, as reported by JOHRI (15) and by DAHLGREN (8, 9), show a reduction in the chalazal part of the embryo sac, which is commonly six-nucleate. The nuclei divide normally up to the four-nucleate stage, after which the chalazal pair remain undivided and the micropylar pair divide once again.

Instead of the three divisions usually found in the *Allium* type, MAGNUS (17) observed only two divisions from the chalazal megaspore of *Podostemon subulatus*. Subsequent studies by HAMMOND (12), WENT (38, 39, 40), and MAHESHWARI (19) question the existence of a *Podostemon* type. SCHNARF (32) likewise questions the megagametophyte described by MAGNUS in *Dicraea elongata*, in which he reported one division of the micropylar dyad, forming the egg and one synergid, and one anticlinal division of the chalazal dyad, forming two antipodal cells. MAGNUS stated that no polar nucleus exists and that consequently double fertilization is impossible.

PACE (26), describing *Cypripedium spectabile*, *C. parviflorum*, *C. pubescens*, and *C. candidum*, found that the megagametophyte was derived from the chalazal dyad. She further observed a four-nucleate megagametophyte resulting from two divisions with a pair of nuclei at each end of the embryo sac. The micropylar nuclei form the two synergids and the two chalazal nuclei form the egg and a single polar nucleus. At the time of fertilization one of the synergid nuclei takes part in triple fusion. PALM (27) established the "*Cypripedium* type" on the

basis of PACE's investigation. RUTGERS (30), in a criticism based primarily upon certain figures in PACE's work, pointed out several weaknesses of the *Cypripedium* type. Recent publications by PROSINA (29), investigating *C. guttatum*, and CARLSON (4), in her study of *C. parviflorum*, show that the development of the megagametophyte is bisporic and of the usual *Allium* type, with imperfect development at the chalazal end so that there are usually only five or six nuclei in the embryo sac. MAHESHWARI (18), in a review of the types of embryo sacs in angiosperms, included a complete list of the investigations on many other genera of the *Allium* type.

Summary

1. The megasporangium of *Allium cernuum* is essentially like that of *Lilium*.

2. The archesporium arises directly from a hypodermal cell about 3 or 4 days before the flowers emerge from the bud.

3. The megagametophyte is formed from the chalazal dyad. The micropylar dyad disintegrates without further division.

4. The chalazal dyad undergoes three divisions, resulting in an eight-nucleate megagametophyte.

5. The mature megagametophyte consists of a large vacuolated egg, two smaller synergids—one of which usually disintegrates before fertilization—two polar nuclei of equal size, and three antipodal cells which may or may not persist.

6. The haploid number of chromosomes in *A. cernuum* is eight.

DE PAUL UNIVERSITY
CHICAGO, ILLINOIS

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FLOWER-BUD FORMATION AND DEVELOPMENT IN MANGIFERA INDICA[1]

MARGARET J. MUSTARD AND S. J. LYNCH

Introduction

Most of the better varieties of mangoes (*Mangifera indica* L.) fail to produce good crops every year. In attempts to overcome this problem, various cultural treatments aimed at inducing flower formation have been employed (**1**, **3**, **8**). SEN and MALLIK (**6**) recently determined the time of flower-bud differentiation for one variety of Indian-grown mango, the Langra, and emphasized the importance of this knowledge in connection with the problem of irregular bearing. SEN (**7**) has utilized this information in preparing a "tentative cultural schedule" as an aid to the more regular production of these fruits.

The present investigation was undertaken to determine the time of flower-bud differentiation and to study the subsequent floral development in some varieties of mangoes grown in southern Florida.

Material and methods

Collections of material were made for two consecutive years from trees of Haden, Brooks, and Cambodiana varie-

[1] This investigation was conducted by the authors while both were employed by the University of Florida at the Sub-Tropical Experiment Station at Homestead, Florida.

ties and a turpentine seedling mango. These four trees are located on soil of the Rockdale series in southern Dade County. Although different parts of the same tree were not always at the same stage of development, an effort was made to select buds which seemed to be representative of the average stage on each date. Collections were started the first year, on September 10, 1943, and were continued at 2-week intervals thereafter until each tree came into bloom. The following year the first collections were made on September 23 and were continued over a similar period.

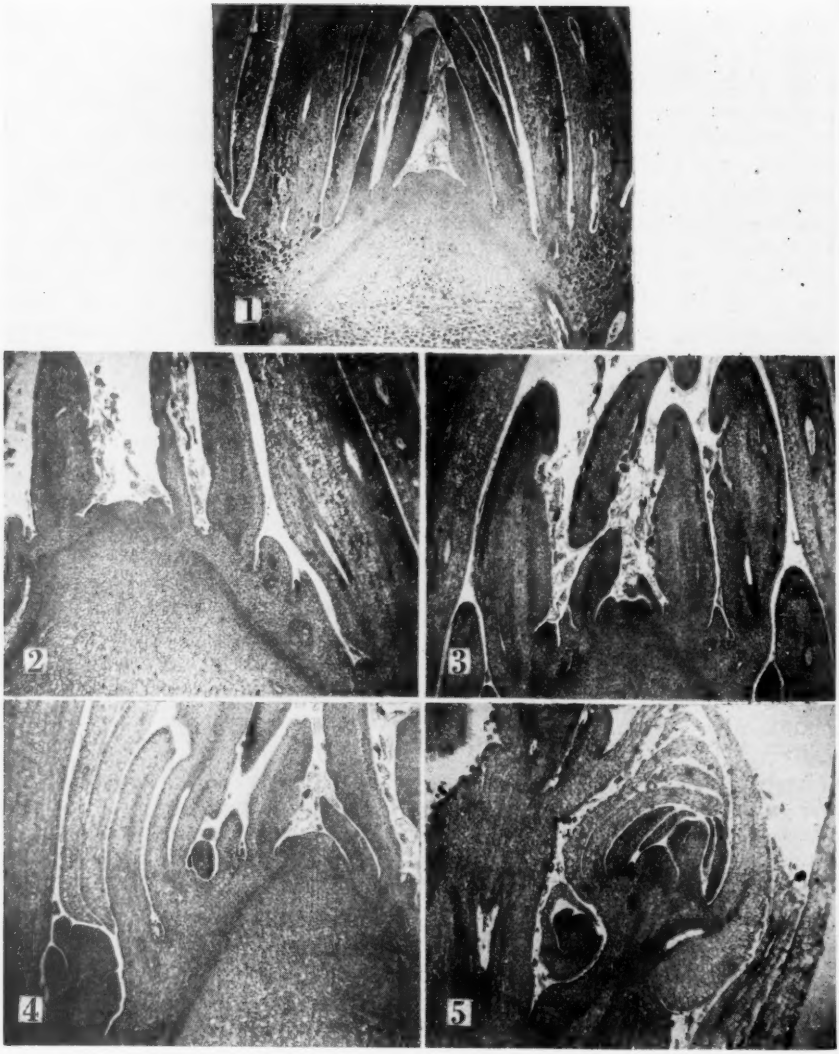
The buds were fixed in F.A.A., dehydrated in tertiary butyl alcohol, and imbedded in paraffin. Before sectioning, the imbedded material was softened for 1–2 weeks in an alcohol-glycerin mixture. Sections were made at 10 μ. Safranin and fast green were used in staining the mounted serial sections.

Observations

A mature mango inflorescence consists of a main axis and a number of profusely branched secondary axes (fig. 6). Each secondary axis, the ramifications of such axes, and the pedicels are subtended by bracts. An individual inflorescence may bear as many as four thousand flowers (**3**), of which the ratio of bisexual to staminate varies with the variety, the bisexual flowers usually being in a decided minority.

Prior to floral differentiation a mango bud consists of a cone of meristematic tissue surrounded by the outer bud scales (fig. 1). The inner bud scales project as finger-like protuberances from the surface of this cone of tissue. The earliest indication of floral development (**6**) is the appearance of mounds of meristematic tissue in the axils of these inner bud scales or bracts (fig. 2). Meristematic areas in the axils of the more proximal bud scales are usually the first to begin floral differentiation. As the season progresses and such growing points enlarge, it becomes obvious that each is the primordium of a secondary axis (fig. 3). Soon these secondary axes give rise to outgrowths of tissue (fig. 4) which prove to be bracts, in the axils of which other small mounds of meristematic tissue develop. These and subsequently differentiated growing-points may follow either of two courses of development: (1) they may differentiate into additional axes, thus continuing the ramification of the inflorescence; or (2), they may differentiate into floral primordia (fig. 5). If such a mound of tissue differentiates into another axis, the process is repeated until a growing-point is formed which differentiates into a flower primordium. It appears that the position of such growing-points on the inflorescence governs to some extent their future course of development, since the greatest degree of ramification occurs in the proximal portions of the lower secondary axes.

In order to record these progressive stages of floral development as they were observed in the sectioned material, a method was employed similar to that described by SELL and his co-workers (**5**). The numerical ratings of 1 through 5 were assigned to each of the progressive stages of development shown in figures 1–5. Using these figures as a basis, it was possible to determine and record numerically the approximate stage of development of an individual bud. Figures for the average stage of development presented in table 1 were obtained by multiplying the number of buds found to be at each of these stages by the assigned numerical rating, adding the products, and dividing this figure by the total num-



FIGS. 1–5.—Longitudinal sections of mango buds. Fig. 1, prior to differentiation. Fig. 2, very early stage of floral differentiation, small protrusion in axil of bud scale appearing at lower right. Fig. 3, slightly more advanced stage, enlarged axillary protrusions or primordia of secondary axes. Fig. 4, early differentiation of bracts on a secondary axis. Fig. 5, arrangement of individual flower parts of hermaphroditic flower.

ber of buds collected on that date. Thus, for example, of the five Haden buds collected on 9/10/43, one was found to be at stage 1 (fig. 1) and four at stage 2 (fig. 2); therefore, the average stage of floral development on that date was 1.8.

It is evident from table 1 that floral differentiation began slightly earlier in the Haden, Cambodiana, and Brooks in 1943 than in 1944; whereas in the turpentine seedling it seems to have occurred at approximately the same time both years. Such variation is to be expected, however, since the time of initiation of floral differentiation is dependent upon a combination of environmental factors and tree condition. These results indicate that by the end of October of both years the majority of the terminal buds studied had shown signs of floral differentiation. Between the time of flower-bud differentiation and that of inflorescence expansion, development seems to have progressed without a period of dormancy such as is common among the deciduous fruit trees of the North Temperate Zone.

FIG. 6.—Mature inflorescence

TABLE 1

AVERAGE STAGE OF FLORAL DEVELOPMENT OF TERMINAL MANGO BUDS. INDIVIDUAL BUDS NUMERICALLY RATED BY COMPARISON WITH FIGURES 1–5

Variety	Average stage of development								
	9/10/43	9/25/43	10/9/43	10/23/43	11/6/43	11/18/43	12/4/43	12/18/43	12/31/43
Haden	1.8	1.8	1.3	1.0	2.0	1.0	2.0	2.0	2.0
Cambodiana	2.0	2.0	2.0	1.5	2.5	2.7	.....	.....	.....
Brooks	1.3	2.0	2.0	2.0	2.3	2.2	3.5	5.0	.....
Turpentine	1.5	2.0	1.5	1.7	3.5	3.0	.....	.....	.....
		9/23/44	10/7/44	10/20/44	11/4/44	11/17/44	12/1/44	12/13/44	12/30/44
Haden	.....	1.0	1.0	1.0	1.3	2.0	2.0	2.7	4.0
Cambodiana	.....	1.0	1.7	1.7	1.5	2.0	1.0	3.3	.....
Brooks	.....	1.0	1.0	2.0	1.7	2.0	2.0	.....	.....
Turpentine	.....	1.5	1.5	2.0	2.7	3.0	4.0	2.0	.....

These observations on the time of floral differentiation agree closely with those made by SEN and MALLIK. These authors found that it occurs shortly after the end of September in the Langra mango grown in India. STURROCK (9) previously estimated the time of floral differentiation as occurring in October or early November in some varieties of Florida-grown mangoes. His collections were too late in the season to determine the early stages of differentiation. REECE (4) observed a similar lack of dormancy between the time of floral differentiation and maturation of flowers in Florida-grown avocados.

Summary

1. Floral differentiation occurred shortly before the end of October in the four varieties of mangoes studied.

2. No period of dormancy occurred between the time of floral differentiation and that of inflorescence expansion.

CHEMISTRY DEPARTMENT
UNIVERSITY OF MIAMI
CORAL GABLES, FLORIDA
AND
FLORIDA LIME AND AVOCADO GROWERS
PRINCETON, FLORIDA

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CURRENT LITERATURE

Pure Cultures of Algae: Their Preparation and Maintenance. By E. G. PRINGSHEIM. Cambridge: At the University Press; New York: Macmillan Co., 1946. Pp. xii+119. $1.75.

For more than a quarter of a century PRINGSHEIM has been the leader in study of algae by pure culture methods. The extensive series of algae which he has isolated include many differing markedly in their growth requirements when grown *in vitro*. In his *Pure Cultures of Algae* he passes along the benefit of his long experience in this field of botanical investigation. His book is not a laboratory manual of the "cookbook" type but is a connected and integrated running account of how to obtain cultures of algae. The reviewer recommends that the person wishing to learn about methods of cultivating algae should begin by reading this small book from cover to cover. By doing this, one gets the concentrated essence of PRINGSHEIM's long experience and thus avoids many pitfalls that would otherwise be encountered the hard way.

The author points out the great advantage of preparatory cultures in which one attempts to gain an insight into the conditions suitable for the existence and multiplication of particular algae. Enrichment (selective) cultures are especially valuable for this purpose, since, by varying the composition of the medium, pH, temperature, and other environmental factors, growth of certain algae in the inoculum is favored, while growth of other algae is checked. Following the chapter on preparatory cultures there are chapters on media, on methods of plating, on isolation by means of micropipettes, and on the maintenance of cultures. The concluding chapter deals with the general conditions essential for growth of members of each of the larger taxonomic groups of algae.

There are some books in which one copy in the departmental library is sufficient. Here is one in which this is not the case; every man who has use for it will want a copy in his personal library.—GILBERT M. SMITH.

Chromosome Atlas of Cultivated Plants. By C. D. DARLINGTON and E. K. JANAKI AMMAL. London: Allen & Unwin, Ltd.; New York: Macmillan Co., 1945. Pp. 397. $2.75.

The chromosome numbers of some ten thousand species of the most useful plants and of their wild relatives and ancestors have been assembled in this world list.

In an introduction, consisting of three parts, Part I deals with the origin of cultivated plants as to (*a*) the foundations as determined especially by VON HUMBOLDT, DE CANDOLLE, DARWIN, and MENDEL; (*b*) the centers of origin according to VAVILOV; (*c*) evolution by cultivation; and (*d*) the invention of plant breeding. Part II introduces the chromosomes, and Part III covers the use and meaning of chromosome numbers.

Next is given an explanation of the catalogue. The families of angiosperms follow the order and bear the numbers of HUTCHINSON's *Families of Flowering Plants*, with 233 of the 332 families represented. Two exceptions to this order are noted. Because of their chromosome affinities, the tribes Allieae and Agapantheae have been retained in the heterogeneous Liliaceae and the Nolanaceae have been kept separate from the Convolvulaceae. The families of gymnosperms, taken from ENGLER and GILG's *Syllabus der Pflanzenfamilien*, follow the author's own order, while the tribes of Pinaceae follow HUTCHINSON's unpublished classification. Five columns are employed in the catalogue. The first column is used for the generic and specific names of some two thousand genera with more than eleven thousand species listed. The second column gives the popular names, the third the chromosome numbers, the fourth the author from whose paper the numbers are reported, and the fifth column the uses to which the plants are put. The catalogue, covering about 309 pages, comes next, followed by a bibliography and by an index to the families and genera.

No attempt is made to cite all the literature dealing with chromosome numbers. Where two accounts differ in number for the same species, both are quoted, apart from certain ancient errors; but, where accounts agree, the last important reference only is quoted, since from this the earlier counts can usually be obtained.

The book is an important contribution and will be highly valuable for teachers and investigators in all phases of economic plant production.—J. M. BEAL.